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LUND 1937, BERLINGSKA BOKTRYCKERIET

DER TOD ALS EIN NUKLEARER PROZESS

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(With a summary in English)

I. WEITERE STUDIEN ÜBER DIE MITOSE.

1. DER START DER MITOSE.

IN meiner Arbeit von 1937 habe ich ganz flüchtig hervorgehoben, dass die in einem keimenden Samen zuerst zu beobachtenden Teilungen stets in der Mitte der Wurzel liegen. Diese Behauptung soll hier des weiteren beleuchtet werden. Zuerst einige Worte über die Methodik bei der Berechnung der in Tab. 1 mitgeteilten Zahlen. Um ganz gleichartige Zahlen erhalten zu können sind nur solche Wurzeln berücksichtigt worden, die die gleiche Anzahl untersuchter Schnitte enthalten (8 St.). Von 1934 B III trocken sind 13 Wurzeln genommen worden, von 1934 B III $1\,^1/_2$ St. 17 Wurzeln, von 1934 B III $3\,^1/_4$ St. 16 Wurzeln. Die entsprechenden Zahlen für 1933 sind 8, 5 und 7, für 1932 7, 6 und 4 sowie für 1931 4, 9 und 10. Von 1934 A V, B V, A IV, B IV trocken, B IV 1 St. sowie B IV 3 St. sind 11, 10, 3, 6, 4 bzw. 2 Wurzeln mit gleichfalls 8 Schnitten per Wurzel genommen worden. Das Material umfasste also insgesamt 142 Wurzeln und gibt ein gutes Bild vom Platze der startenden Kerne in der Wurzel. In sämtlichen Wurzeln sind die Teilungen der zwei äussersten Schnitte für sich addiert worden und daraus ist der Prozent »peripherer» Zellen in den verschiedenen Stadien des Keimungsprozesses berechnet worden.

Zuerst nun die B III-Serien. Im Gebiete 0—10 Teil./Schnitt haben die repräsentierten Wurzeln 0, 8,2, 6,7 % periphere Zellen, im Gebiet 10—20 Teil./Schnitt 22,4, 17,2, 10,3, 22,5, 15,2, 19,4 und 19,7 %. Im ersten Stadium des Keimungsprozesses nehmen also ausschliesslich oder hauptsächlichst zentrale Zellen teil. Unmittelbar danach greift der Teilungsprozess auf grössere Teile der Wurzel über, und im Gebiet 20—30 Teil./Schnitt wird das Maximum von peripheren Zellen erreicht, das dann durch die hier repräsentierten Gebiete konstant gehalten wird. Die B IV- und die A V-Serien bestätigen dies. Hier enthält das 0—20-Gebiet 16,3 bzw. 16,1 %. (Es sei jedoch hinzugefügt, dass eine Wurzel von 1934 B IV im 0—10-Gebiet 23,2 % periphere Zellen enthielt.) Das 20—

TABELLE 1. *Die Prozentzahl von peripheren Teilungen in verschiedenen Teilungsgebieten.*

Serie	Die Anzahl von Teilungen per Schnitt							Anzahl der Teilungen
	0 — 10	10 — 20	20 — 30	30 — 40	40 — 50	50 — 60	60 — 70	
1934 B III trocken.....	0(4)	22,4(532)[1]	23,2(668)	19,6(1081)	22,6(1005)	—	—	3290
1934 B III 1 1/2 St.	—	15,2(79)	19,2(380)	24,4(2242)	17,3(1131)	22,5(1209)	—	5041
1934 B III 3 1/4 St.	—	28,5(151)	17,2(594)	19,1(1652)	18,0(2134)	—	—	4531
1933 B III trocken.....	—	17,2(268)	19,4(520)	19,5(765)	—	—	—	1553
1933 B III 1 1/2 St.	—	—	18,6(603)	24,2(537)	18,2(319)	—	—	1459
1933 B III 3 1/4 St.	—	28,9(152)	20,0(401)	22,3(1044)	—	—	—	1597
1932 B III trocken.....	—	10,3(78)	—	21,5(842)	23,1(971)	—	—	1891
1932 B III 1 1/2 St.	6,7(15)	19,4(98)	21,1(356)	17,9(554)	—	—	—	1023
1932 B III 3 1/4 St.	—	—	—	21,7(1075)	—	—	—	1075
1931 B III trocken.....	8,2(49)	22,5(89)	25,4(184)	26,1(268)	—	—	—	590
1931 B III 1 1/2 St.	—	19,7(218)	20,8(995)	22,4(585)	—	—	—	1798
1931 B III 3 1/4 St.	—	19,9(377)	21,9(602)	24,4(1124)	—	—	—	2103
1934 A V.....	—	16,1(124)	22,0(191)	21,9(859)	23,7(758)	23,0(936)	20,4(1046)	3914
1934 B V.....	—	—	20,9(239)	23,9(1423)	22,7(1089)	22,2(419)	—	3170
1934 A IV trocken	—	—	—	20,1(293)	—	22,4(823)	—	1116
1934 B IV trocken	16,3(147)		—	—	20,9(793)	16,0(469)	20,3(1523)	2932
1934 B IV 1 St.	—	—	—	—	—	21,5(876)	18,8(1008)	1884
1934 B IV 3 St.	—	—	—	—	24,5(371)	—	19,1(530)	901

[1] Die Parenthesen zeigen die absolute Anzahl der Teilungen.

30-Gebiet enthält bei A V und B V 22,0 bzw. 20,9 % periphere Zellen; auch diese Zahlen sind also niedriger als in den darauffolgenden Klassen. In den höchsten Klassen (mit mehr als 60 Teil./Schnitt), bei 1934 B V auch in der 50—60-Klasse, besteht eine Tendenz zu Abnahme der Anzahl peripherer Zellen. Bei 1934 A V liegt das Gebiet 60—70 erheblich unter dem vorherigen (mit 2,6 %), bei 1934 B IV 1 St. und 1934 B IV 3 St. gleichfalls (mit 2,7 bzw. 5,4 %). 1934 B IV trocken bildet eine Ausnahme, aber dies dürfte darauf beruhen, dass die Anzahl peripherer Zellen in der Klasse 50—60 unter normal ist.

Wir erhalten also folgendes schematisches Bild: Zentrale Zellen beginnen mit der Teilung, darauf nehmen die peripheren an Anzahl zu, der Teilungsstrom nimmt zu, aber nachdem die Anzahl der peripheren Zellen eine zeitlang sich in maximaler Lage befunden hat, nimmt sie ab. Letzteres beruht nicht darauf, dass vom Start der Keimung bis zum Fixierungsaugenblick längere Zeit verflossen ist, sodass die ersten in Teilung befindlichen Zellen sich noch einmal haben teilen können. Sämtliche Wurzeln der IV- und V-Serien sind nämlich 48 Stunden nach dem Auslegen zum Keimen fixiert worden. Anstatt dessen dürfte dies darauf beruhen, dass der Wassergehalt der Ruhekerne nicht in der ganzen Wurzel vollkommen gleichartig ist, sondern sowohl nach einer Plus- wie Minusrichtung verschoben erscheint, wobei die zentralen Teile die grösste Variation aufweisen. Diese diskontinuierliche Verteilung der Wassermenge wird übrigens schon dadurch bewiesen, dass die ersten Kerne in Teilung zentral belegen sind.

Die Ursache der zentralen Lage der ersten Teilungen muss wenigstens teilweise nuklear-chromosomal sein. Die startenden Kerne liegen näher dem Reproduktionspunkt. Die Chromosomen in denselben sind mehr oder weniger auf die Teilung vorbereitet. Wenn die Wasserzufuhr für eine vollständige Reproduktion hinlänglich geworden ist, beginnt die Teilung in diesen Kernen zuerst.

In bezug auf die eigentümlichen Zahlen der 3 1/4 St. befeuchteten Serien siehe S. 29.

2. DER ÜBERGANG ZWISCHEN RUHESTADIUM UND PROPHASE.

Laut der Zwei- und Vierfadenauffassung sind die Veränderungen, die die Chromosomen beim Übergang in die Prophase erleiden, nur von gradueller Natur. Veränderungen in Spiralisation und Kontraktion, im Wassergehalt, in der Mächtigkeit der Kalymna bedeuten keine Strukturveränderungen im Inneren der Chromosomen. Die Einfadenauffassung behauptet anstatt dessen einen bestimmten Unterschied zwischen Ruhe-

stadium und Prophase, es bestehe zwischen diesen sozusagen eine Wand, bedingt durch die Genen- und Fibrillenreproduktion. Untersuchungen über den Teilungsrhythmus nach Einwirkung von Narkotika oder Röntgenstrahlen bestätigen die Richtigkeit der Einfadenauffassung und legen den Zeitpunkt für den Übergang zwischen Ende des Ruhestadiums und Beginn der sichtbaren Prophase klar.

Die auftretenden Störungen bei Giftbeeinflussung oder Bestrahlung mit X-Strahlen und α-Partikelchen können in drei Gruppen eingeteilt werden: 1) der sog. Primäreffekt (POLITZER, 1934), bestehend in einer Pyknotisierung der Chromosomen und der Bildung von Pseudoamitosen, 2) eine mitosenfreie Zwischenzeit und 3) der sog. Sekundäreffekt, bestehend in einer Fragmentation der Chromosomen sowie Fusionierung von Chromosomenenden ohne Pyknotisierung. Diese drei Gruppen müssen genau auseinander gehalten werden. Wie POLITZER mit Recht hervorhebt, beruhen die abweichenden Resultate in vielen Untersuchungen darauf, dass die drei Prozesse nicht auseinander gehalten worden sind. Organische Gifte und Narkotika verursachen in gewissem Masse spezifische Störungen, sie zeigen nicht den oben genannten Sekundäreffekt, der nur durch den Einfluss von Röntgenstrahlen u. dgl. entsteht, sie zeigen jedoch den Primäreffekt obgleich ohne Pyknotisierung der Chromosomen sowie vor allem *den mitosenfreien Zeitraum.* Diesen haben sämtliche störenden Agentien gemeinsam.

Der Primäreffekt ist *unmittelbar* nach der Bestrahlung oder der Applizierung des Giftstoffes wahrnehmbar und besteht bei starker Bestrahlung und nachfolgender Pyknotisierung der Chromosomen in einer Auflockerung der Chromosomenhülle (der Kalymna), sodass die verschiedenen Chromosomen sowohl lateral wie terminal zu einer diffusen Masse zusammenschmelzen können. Diese Pyknotisierung ist also mit der Fusionierung, die beim Sekundäreffekt auftritt, nur oberflächlich verwandt: Die sekundären Fusionen werden allerdings mit den Enden so stark verschmolzen, dass die letzteren nur selten voneinander losgelöst werden können, da aber die Kalymna bei ihrer Entstehung (dem Start der Prophase) gerade sich zu bilden beginnt, kann die Verschmelzung nicht auf Veränderungen in derselben beruhen.

Die mitosenfreie Periode entsteht nach und nach wie die in Teilung befindlichen Kerne die Teilung vollziehen oder degenerieren. Es zeigen also nur jene Kerne einen Primäreffekt, die bei der Bestrahlung etc. sich in Teilung befunden haben. Die mitosenfreie Periode ist je nach der Intensität der Bestrahlung oder der Giftwirkung verschieden lang.

Sie beginnt indessen stets mit jenen Kernen, die gerade ihre Kernteilung gestartet haben sollten.

Das mitosenfreie Stadium bei Röntgenbestrahlung beruht laut darauffolgender Untersuchung auf einem Absterben der Ruhekerne, die im oder zunächst dem Reproduktionsstadium sich befinden, nicht nur jener Kerne, die gerade die Reproduktion ausführen, obgleich diese am empfindlichsten sind. Bei Narkotisierung dagegen beruht der Stillstand in der Teilung wahrscheinlich auf dem Tod der Kerne, die sich gerade in Reproduktion befinden, und auf dem Unvermögen der benachbarten Kerne die Reproduktion durchzuführen bevor das Gift unschädlich gemacht oder aus dem Kern entfernt worden ist, sodass der komplizierte Reproduktionsmechanismus wieder fungieren kann. BECKERS Experimente mit Vitalfarbstoffen (1932) zeigen die Bedeutung des *Kerns* bei der Giftwirkung. Bei starker Färbung wird der Vitalfarbstoff in den Kern aufgesaugt. Gleichzeitig machen sich schwere funktionelle Störungen in der Zelle geltend. Aber je mehr das Gift aus dem Kern entfernt wird, umso leichter gesundet die Zelle.

Hier sei über einige Narkotisierungsexperimente näher berichtet, die POLITZER mit Larven von *Salamandra* ausgeführt hat. Die Larven wurden einer 4-stündigen Narkotisierung mit 5 %-igem Alkohol unterzogen. Hierauf wurden sie wieder in Wasser gebracht, gesundeten aber nach der Narkose nicht sondern gingen nach 12—24 Stunden zugrunde. Die sechs Figuren veranschaulichen die Häufigkeit der verschiedenen Teilungsstadien nach verschiedenen Fixierungszeiten. *a* gibt das Stadium an, in dem der Kern der Prophase sich von einem Ruhekern durch die beginnende Differenzierung des Chromatins trennt, *b* die spätere Prophase, *c* das Metaphasenstadium, *d* die frühe Anaphase, *e* die späte Anaphase, *f* die frühe Telophase und *g* die späte Telophase. Die Fixierungen sind 2, 3, 4, 7, 8 bzw. 10 Stunden nach Beginn der Narkose ausgeführt. Nach 2 Stunden hat die Anzahl Prophasen und die Anzahl Ana-Telophasen bereits abgenommen, nach 3 Stunden ist nur eine geringe Anzahl von Prophasen und eine geringe Anzahl von Ana-Telophasen vorhanden, i. ü. gibt es nur Metaphasen. Das gleiche Verhältnis findet man nach 4 Stunden. Nach 7 Stunden sind die Prophasen vollständig verschwunden und die Anzahl Metaphasen hat abgenommen während die Ana- und Telophasen wieder zuzunehmen beginnen. Dies ist noch ausgeprägter nach 8 Stunden und nach 12 Stunden kommen fast ausschliesslich Ana- und Telophasen vor.

Die Narkotisierung hindert also Ruhezellen daran, in die Teilung einzutreten, auch hindert sie den Beginn der Anaphasenbewegung, also

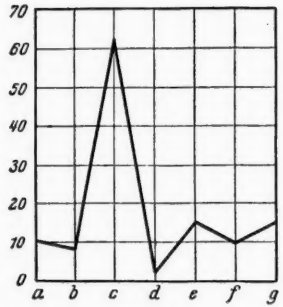

Fig. 1.

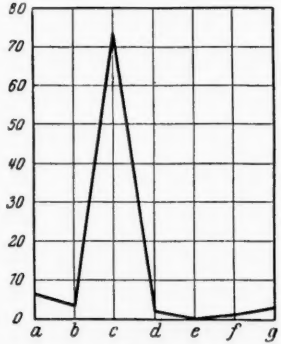

Fig. 2.

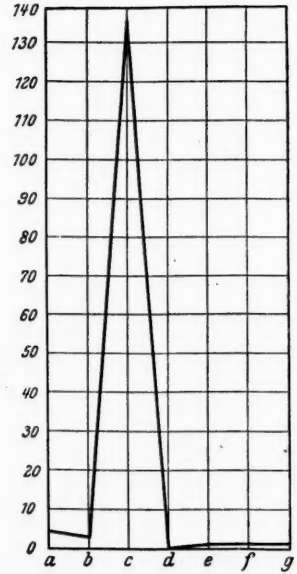

Fig. 3.

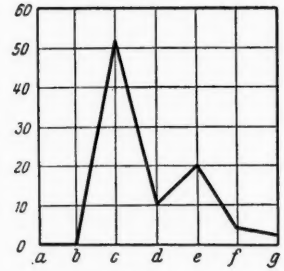

Fig. 4.

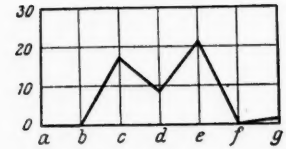

Fig. 5.

Fig. 6.

Fig. 1—6. Der Abstand zwischen Reproduktionsstadium und Prophase durch die Teilnahme der verschiedenen Phasen der Mitose 2, 3, 4, 7, 8 und 10 Stunden nach dem Beginn einer tötenden Narkotisierung veranschaulicht. Die wenigen Prophasen in Fig. 6 zeigen, dass die Kerne unmittelbar in der Nähe vom Reproduktionspunkt sehr verzögert werden. (Aus POLITZER, 1934.)

einerseits die Reproduktion der Chromonemafäden, andererseits die Repulsion zwischen den Constrictions, die laut DARLINGTONS Bewegungstheorie in Prometaphase-Metaphase geteilt werden. Mit grösster Schärfe hebt POLITZER (S. 124) hervor, dass nach den in Teilung begriffenen Zellen keine weiteren Ruhezellen mit der Teilung beginnen können. Den Zeitpunkt für die Reproduktion glaube ich unmittelbar vor die sichtbare Prophase verlegen zu können. Zwischen der Reproduktion und der sichtbaren Prophase besteht daher im oben genannten Experiment so gut wie keine Zwischenzeit. Natürlich kann der Übergang zwischen Ruhestadium und Prophase bei verschiedenen Arten oder verschiedenen Organen verschiedene Dauer haben. Auch die rein physikalischen Prozesse, die nach der Reproduktion stattfinden, nehmen ja auch etwas Zeit in Anspruch.

II. DIE VERÄNDERUNGEN DER CHROMOSOMEN UND DER NATUR DER MUTATIONEN BEIM ALTERN.

1. VERÄNDERUNGEN DER CHROMOSOMEN MIT DEM ALTERN.

In meinen früheren Arbeiten habe ich eingehend über die Gesetzmässigkeiten bei der Entstehung der zwei Haupttypen von Störungen berichtet (Translokationen als Folge von Fragmentation mit darauf folgender Fusionierung haben in meinem Material nicht analysiert werden können). — Die Stärke der Fusionierung ist eine Funktion der Intensität der Röntgenbestrahlung sowie des Alters der Zellkerne, während die Fragmentation eine Funktion von Wassergehalt, der Intensität der Bestrahlung und des Alterns ist. Die Fusionierung habe ich als einen (physikalisch-) chemischen Prozess, beruhend auf Veränderungen in den Chromosomen*enden*, gedeutet (HUSKINS und HUNTERS Resultate, 1935, hinsichtlich der Frequenz der lateralen Fusionen können nicht generalisiert werden). Die Fragmentation dagegen bedeutet eine Zersplitterung, die in den Reproduktionsstadien der Chromosomen zunimmt (ein biologischer Prozess).

Tab. 2 bringt eine Zusammenfassung der 1936, S. 503 publizierten Resultate und veranschaulicht schematisch die Veränderungen in der Frequenz von Fusionen und Fragmentationen der drei Jahrgänge 1934—1932 B III im Zusammenhang mit einer Zunahme des Wassergehalts. Die starke Zunahme der Fragmentation im 0—40-Gebiet ist trotz der verminderten Bestrahlung deutlich, während die Anzahl der Fusionen wesentlich abnimmt. Im 40—60-Gebiet besteht ein gerade entgegengesetztes Verhältnis: Die Stärke der Fragmentation ist vermindert, die

Fusionierung stark erhöht oder ein wenig vermindert. Eine Vereinigung der drei Jahresklassen gibt das gleiche Resultat. Die Bedeutung dieser Erscheinung habe ich früher klargelegt.

Das Altern bedeutet eine gradweise Veränderung der Chromoso-

TABELLE 2. *Die relative Anzahl der Zellen mit Fusionen und Fragmenten in den B III-Serien* (*s.* GUSTAFSSON, *1936, p. 503*).

Serie	Zellen mit Fusionen						Zellen mit Fragmenten					
	Die Anzahl Teilungen per Schnitt											
	0	—		40(30)	—	60	0	—		40(30)	—	60
	1934	1933	1932	1934	1933	1932	1934	1933	1932	1934	1933	1932
trocken	1	1	1	1	1	1	1	1	1	1	1	1
1 ½ St.	0,83	0,75	0,73	1,16	1,03	1,24	0,98	0,86	1,15	0,84	0,77	0,76
3 ¼ St.	0,43	0,33	0,32	1,42	0,76	0,90	1,23	1,10	1,17	0,73	0,93	0,87
	Σ 1934—1932			Σ 1934—1932			Σ 1934—1932			Σ 1934—1932		
trocken	1			1			1			1		
1 ½ St.	0,76			1,09			1,00			0,81		
3 ¼ St.	0,37			1,04			1,17			0,82		

TABELLE 3. *Die Verteilung der verschiedenen B III trocken- und 1 ½ St.-Wurzeln auf verschiedene Störungsgebiete* [1].

Serie	Prozentzahl der gestörten Teilungen										
	0	10	20	30	40	50	60	70	80	90	100
1934 trocken	4	12	5	1	—	—	—	—	—	—	—
1933 »	—	3	4	2	2	—	—	—	—	—	—
1932 »	—	—	5	1	1	1	2	1	—	—	—
1931 »	—	—	—	—	—	1	2	1	1	—	—
1934 1 ½ St.	8	12	—	1	—	—	—	—	—	—	—
1933 »	5	4	3	3	1	—	—	—	—	—	—
1932 »	1	3	3	1	2	1	—	—	—	—	—
1931 »	—	—	1	4	4	—	—	—	1	—	—

men. 1937 Tab. 17 und 20 zeigen dies. Infolge eines Zufalls (?) verhalten sich die Störungsprozente der vier Jahrgänge wie 1 : 2 : 3 : 4,5. NAVASHIN und Mitarbeiter haben in einer Serie von Arbeiten den Einfluss des Alterns auf die Frequenz von Chromosomenmutationen unter-

[1] Nur die Wurzeln sind mitgenommen, welche mehr als 10 Teilungen per Schnitt enthalten.

sucht, behaupten aber eine diskontinuierliche Zunahme. Es erscheint selbstverständlich, dass beim Altern von Samen Chromosomenstörungen erst dann entstehen können, wenn die Zellen dem Tode so nahe gekommen sind, dass die Kerne abnorm geworden sind. Die Diskontinuität ist daher nur eine scheinbare. Die Bestrahlung entschleiert die gradweise Veränderung der Chromosomen. Diese geht auch aus Tab. 3 hervor. Die Variation in der Störungsfrequenz zwischen den verschiedenen Wurzeln steigt von 1934—1932 um in den Samen des Jahres 1931 wieder abzunehmen, wo die Empfindlichkeit für die Bestrahlung so stark geworden ist, dass nur eine unbedeutende Variation besteht. Verschiedene Wurzeln altern also ungleich schnell.

TABELLE 4. *Die Prozentzahl von Zellen mit Fusionen und Fragmenten.*

Serie	% Zellen mit Fusionen						% Zellen mit Fragmenten					
	1934	1933	1932	1934—1932	1931	D_1	1934	1933	1932	1934—1932	1931	D_2
trocken	49,0	60,1	49,0	51,1	60,4	9,3	68,6	74,7	72,4	71,8	78,3	6,5
1 1/2 St.	44,8	46,9	46,2	46,0	50,9	4,9	64,1	62,5	72,4	66,2	74,2	8,0
3 1/4 St.	44,6	25,0	40,4	38,5	47,9	9,4	64,9	77,5	66,0	68,3	68,1	—0,2
Σ D						23,6	.....					14,3

Die zwei Störungstypen nehmen mit zunehmendem Alter ungefähr gleich schnell zu. Die relativen Zahlen für die Trockenserien betragen für die Fragmentationen 1 : 1,8 : 2,9 : 5,0 und für die Fusionen 1 : 2,0 : 2,7 : 5,4. Der Unterschied in der Empfindlichkeit zwischen 1932 und 1931 ist frappant. Der biologische Prozess, der eine Zunahme der Fragmentanzahl verursacht, nimmt an Stärke in gleichem Grade zu wie der chemische Prozess, der die Fusionierung bedingt. Ein gewisses Übergewicht für die Fusionen ist jedoch bemerkbar. Das Verhältnis zwischen Zellen mit Fusionen oder Fragmenten zur Totalanzahl Zellen mit Störungen in den verschiedenen Serien geht aus Tab. 4 hervor. Die Unterschiede zwischen dem Durchschnitt für einerseits 1934—1932 und andererseits 1931 betragen in den drei Parallelserien für die Fragmentation 6,5, 8,0 und — 0,2 %, für die Fusionierung 9,3, 4,9 und 9,4 %. Die totalen Unterschiede betragen 14,3 % gegen 23,6 %, d. h. eine relative Zunahme der Fusionen mit beinahe dem Doppelten.

Die Störungsfrequenz steigt nicht nur hinsichtlich der *Anzahl ge-*

störter Zellen von 1934—1931 sondern auch in bezug auf die *Anzahl der Störungen in den gestörten Zellen.* Die Anzahl voneinander vollkommen freier (doppelter oder ausnahmsweise einfacher) Fragmente pro gestörter Zelle beträgt für 1934 1,21, für 1933 1,17, für 1932 1,45 und für 1931 1,88, d. h. die Zahl für 1931 ist 1,6 Mal so gross als die für 1934. Die entsprechenden Zahlen für die voneinander freien Fusionen betragen für 1934 1,03 per gestörte Zelle, für 1933 1,07, für 1932 1,11 und für 1931 1,20. Die Zunahme ist hier 1,2-fach. Die Anzahl möglicher Fusionen innerhalb desselben Kerns ist indessen begrenzt (nur 7 mögliche end-to-end Fusionen), während die Anzahl Fragmente unbegrenzt ist. Wie oben gezeigt worden ist, steigt die Anzahl Zellen mit Fusionen mit zunehmendem Alter etwas stärker als die der Zellen mit Fragmenten.

Die Entstehung von Fusionen enthält zwei Probleme: Wie und weshalb erfolgt eine Verschmelzung zwischen Chromosomen*enden*? Weshalb wird die Verschmelzung so stark dass die Fusion sich wie eine Einheit benimmt und an der Verschmelzungsstelle nicht leichter gebrochen wird als an anderen Stellen?

Die Gene liegen linear in den Chromosomen, Seitenketten können nicht entstehen oder sind solchenfalls ohne Evolutionswert. Dies dürfte wahrscheinlich darauf beruhen, dass Gene oder Genkonglomerate (Chromomeren) untereinander eine terminale Attraktion besitzen. Dass dies wirklich der Fall ist, scheint mir gerade durch die Entstehung der terminalen Fusionen bewiesen zu werden. Der Prozess, wodurch sie entstehen, muss auf terminale chemische Kräfte beruhen (freie Valenzen oder dgl.) und diese werden aktiviert oder entstehen teils durch die Röntgenbestrahlung, teils durch das Altern. Dass die Fusion sich nach der Entstehung jetzt *wie eine Einheit* verhält, die zufolge der Repulsion zwischen den Constrictions mitunter auseinander gezogen werden kann, ist nicht merkwürdiger als dass z. B. die Glykoseanhydriden in der Zellulose gleichartig und die Bindungen zwischen denselben gleich sind.

Die Entstehung von Chromosomen aus den freien Genen oder Genhaufen, die bei Blaualgen u. a. vorkommen, dürfte daher in der einen oder anderen Weise durch Kräfte stattgefunden haben, die jenen gleichen, die bei der Bildung von terminalen Fusionen wirksam sind.

1936, S. 502 und Tab. 9 habe ich über das abweichende Verhalten der 1931-er Samen in bezug auf das Verhältnis zwischen Fragmenten und Fusionen in den verschiedenen Stadien des Keimungsprozesses berichtet. Sowohl in der $1\,^1/_2$ St.- wie in der $3\,^1/_4$ St.-Serie ist die Fragmentation im 0—20-Gebiet stark vermindert und eine entsprechende Zunahme der Fusionen findet statt. In den 20—30- und den 30—40-

Gebieten ist die Fragmentation dagegen relativ betrachtet (1936, Tab. 5) wieder erhöht. Die starke Abnahme der Fragmentation ist zu gross um durch den Zufall allein erklärt zu werden, desgleichen ist die starke Zunahme der Anzahl Fusionen statistisch sicher.

Die Samen von 1934, 1933 und 1932 unterscheiden sich also mit Bestimmtheit von denen des Jahres 1931. Das Altern bringt mit sich, dass bei Wasserbehandlung von Karyopsen von 1931 die Kerne, *die zuerst in Teilung treten,* abnorm starke physiologische Veränderungen in den Chromosomenenden aufweisen oder dass die Chromosomen in diesen Kernen zu Beginn der Prophase so langsam voneinander ziehen, dass längere Zeit für das Verschmelzen zur Verfügung steht. Von grossem Interesse ist hierbei, dass gerade in den Samen von 1931 sowohl in der Trocken- wie in der $1\,^1/_2$ St.-Serie (entsprechende Zahlen für die $3\,^1/_4$-St.-Serie fehlen) die relative Teilungsgeschwindigkeit im 0—10-Gebiet, d. h. in den allerersten Teilungen, abnorm niedrig ist. Dass die geringe Teilungsgeschwindigkeit nicht allein die Ursache der Zunahme in der Anzahl Fusionen ist, geht indessen daraus hervor, dass 1931 trocken keine abnorme Fusionszunahme aufweist und dass die gleiche Serie nur eine Veränderung der Teilungsgeschwindigkeit im 0—10-Gebiet zeigt, desgleichen dass die Anzahl Fragmentationen bei 1931 $1\,^1/_2$ St. und $3\,^1/_4$ St. auch im 20—30-Gebiet im Vergleich mit der Trockenserie vermindert ist (1936, Tab. 9). Die Gesamtmenge Fusionen steigt wesentlich mit dem Alter der Samen, aber die Veränderung, die zu einer grösseren Menge von Fusionen führt, ist in den ersten Stadien des Keimungsprozesses am stärksten ausgeprägt. Die ersten Kerne in Teilung sind nahe dem Tode gewesen.

Das Verhalten der ersten Teilungen in Samen von hohem Alter, die nicht dem Einfluss äusserer Agentien ausgesetzt sind, dürfte den endgültigen Beweis für die Richtigkeit dieser Auffassung erbringen. Eine solche Untersuchung ist bereits von NAVASHIN und GERASSIMOVA (1935) ausgeführt worden. Sie haben die Entstehung von Chromosomenmutationen in auf natürlichem Wege gealterten Samen festgestellt. Aber diese Chromosomenveränderungen sind ausschliesslich an das erste Stadium des Keimungsprozesses gebunden. »Mutations arise in resting nuclei and are manifested in the *very first cell-divisions* when the embryo sets for germination». Diese vom Standpunkte der beiden Verfasser unerklärliche Tatsache schliesst die Kette in diesen und später vorgelegten Tatsachen. — Der Tod erfolgt in Kernen, die wegen der Nähe des Reproduktionspunktes die stärksten Störungen im Reproduktionsmechanismus erleiden.

2. DER EINFLUSS VON X-STRAHLEN AUF WURZELN IN DER GLEICHEN KARYOPSE.

In meiner Arbeit von 1937 habe ich nachgewiesen, dass die Chromosomen in jenen Kernen, die zuerst mit der Teilung in einer Wurzel beginnen, von der Bestrahlung stärker beeinflusst werden als Kerne, die sich später teilen. In Tabelle 3 oben genannter Arbeit ist das ganze untersuchte Material vorgelegt worden. Von einer grossen Anzahl von Karyopsen ist mehr als eine Wurzel untersucht worden. Wie verhalten sich da diese Wurzelpaare? Die zytologische Ungleichwertigkeit zwischen den Zellkernen einer Wurzel verlangt, das jene Wurzel eines Wurzelpaares, die die geringste Anzahl Teilungen per Schnitt hat, meistens den grössten Störungseffekt aufweisen soll. Dass dies auch zutrifft, zeigen die unten folgenden Zusammenstellungen.

Die Zusammenstellungen sind in drei verschiedenen Weisen ausgeführt worden: 1) Bei *sämtlichen* Wurzelpaaren ist die Übereinstimmung mit dem erwarteten Verhältnis kontrolliert worden. In den Fällen wo von einer Karyopse mehr als zwei Wurzeln untersucht worden sind, sind die zwei Wurzeln mit der niedrigsten und der höchsten Störungsfrequenz berücksichtigt worden. 2) Nur jene Wurzelpaare, deren Wurzeln 5 oder mehr als 5 % Unterschied in der Teilungsfrequenz per Schnitt aufweisen, sind berücksichtigt worden. 3) Nur jene Wurzelpaare, deren Wurzeln 5 oder mehr als 5 % Unterschied in der Störungsfrequenz zeigen, sind berücksichtigt worden. — Im zweiten und dritten Fall sind von Karyopsen mit mehr als zwei untersuchten Wurzeln jene zwei berücksichtigt worden, die den grössten Unterschied in der Anzahl Teilungen per Schnitt bzw. Störungsfrequenz zeigen. In der ersten Karyopse von 1931 B III trocken sind in der dritten Zusammenstellung die zwei Wurzeln mit 71 und 82 % Störungen berücksichtigt worden.

In Tab. 5 bedeutet ein Plus, dass die Wurzel eines Wurzelpaares, die die niedrigste Teilungszahl per Schnitt hat, auch die höchste Störungsfrequenz aufweist, ein Minus dagegen dass die Wurzel mit der niedrigeren Teilungszahl per Schnitt den niedrigeren Störungsprozent hat.

Von den 131 untersuchten Wurzelpaaren zeigen 73 +, 58 —, während das erwartete Verhältnis 65,5 : 65,5 wäre, falls die Störungsfrequenz vom Teilungsstadium, in dem die Wurzel sich befindet, vollkommen unabhängig sein würde.

Werden die verschiedenen Jahrgänge für sich studiert, so zeigt sich, dass 1931 sowohl in der Trocken- wie in den 1 $^1/_2$ St.- und 3 $^1/_4$ St.-Serien

von den drei anderen Jahrgängen abweicht. Für den Unterschied zwischen 1934—1932 trocken und 1931 trocken beträgt χ^2 2,900, für den Unterschied zwischen 1934—1932 1 $^1/_2$ St. und 1931 1 $^1/_2$ St. 4,015. (Unterschiede, wo χ^2 grösser als 3,841 ($P < 0{,}05$) ist, werden als sicher betrachtet. Wo χ^2 grösser als 6,635 ist, wird P niedriger als 0,01). Werden die Trok-

TABELLE 5. *Die Wirkung der Röntgenbestrahlung auf die Wurzeln innerhalb derselben Karyopse.*

1.

Serie	+	−	Serie	+	−	Serie	+	−
1934 A + B trocken	28	20	1934 B 1—1 $^1/_2$ St.	12	5	1934 B 3—3 $^1/_4$ St.	5	7
1933 A + B »	10	5	1933 B 1 $^1/_2$ St....	1	6	1933 B 3 $^1/_4$ St....	4	1
1932 B »	4	3	1932 B » ...	2	2	1932 B » ...	2	2
1934—1932 »	42	28	1934—1932 » ...	15	13	1934—1932 » ...	11	10
1931 B »	0	2	1931 B » ...	0	4	1931 B » ...	5	1

2.

Serie	+	−	Serie	+	−	Serie	+	−
1934 A + B trocken	15	11	1934 B 1—1 $^1/_2$ St.	7	4	1934 B 3—3 $^1/_4$ St.	2	4
1933 A + B »	7	3	1933 B 1 $^1/_2$ St....	1	4	1933 B 3 $^1/_4$ St....	3	1
1932 B »	4	1	1932 B » ...	2	1	1932 B » ...	1	0
1934—1932 »	26	15	1934—1932 » ...	10	9	1934—1932 » ...	6	5
1931 B »	0	2	1931 B » ...	0	3	1931 B » ...	2	0

3.

Serie	+	−	Serie	+	−	Serie	+	−
1934 A + B trocken	10	5	1934 B 1—1 $^1/_2$ St.	5	1	1934 B 3—3 $^1/_4$ St.	1	0
1933 A + B »	6	2	1933 B 1 $^1/_2$ St....	0	2	1933 B 3 $^1/_4$ St....	0	0
1932 B »	2	1	1932 B » ...	2	1	1932 B » ...	1	1
1934—1932 »	18	8	1934—1932 » ...	7	4	1934—1932 » ...	2	1
1931 B »	0	1	1931 B » ...	0	2	1931 B » ...	2	1

ken- und die 1 $^1/_2$ St.-Serien vereinigt, was wegen ihres gleichartigen Verhaltens motiviert erscheinen kann, so erreicht der Unterschied zwischen 1934—1932 und 1931 für $\chi^2 = 7{,}730$. Der Unterschied zwischen 1934—1932 3 $^1/_4$ St. und 1931 3 $^1/_4$ St. erreicht keinen signifikativen Wert. Wahrscheinlich weichen indessen die Karyopsen von 1931 von den drei anderen in sämtlichen drei verschieden behandelten Serien ab.

Werden die drei Serien von 1931 untereinander verglichen, so zeigt sich, dass die Unterschiede zwischen 1931 trocken und 3 $^1/_4$ St. und zwischen 1931 1 $^1/_2$ St. und 3 $^1/_4$ St. für $\chi^2 = 4{,}441$ bzw. $6{,}667$ betragen. Werden 1931 trocken und 1 $^1/_2$ St. (vgl. oben) vereinigt, so erreicht χ^2 zwischen diesen und 3 $^1/_4$ St. $8{,}571$.

Die zweite Zusammenstellung ergibt eine Summe von 44 Wurzeln mit + und 34 mit —. Der Unterschied zwischen den 1934—1932 Trocken- und den 1 $^1/_2$ St.-Serien einerseits sowie den entsprechenden Serien von 1931 andererseits gibt für χ^2 $6{,}728$. Der Unterschied zwischen den 1931 Trocken- und den 1 $^1/_2$ St.-Serien sowie der 1931 3 $^1/_4$ St.-Serie ergibt für χ^2 $7{,}021$.

Die dritte Zusammenstellung, die am verlässlichsten sein dürfte (ausser in bezug auf die 1931-Serien wegen der hier mangelhaften Repräsentation), zeigt 29 Wurzeln mit + und 17 Wurzeln mit —. χ^2 erreicht für den Unterschied zwischen 1934—1932 trocken + 1 $^1/_2$ St. und 1931 trocken + 1 $^1/_2$ St. $5{,}367$, sowie für den Unterschied zwischen 1931 trocken + 1 $^1/_2$ St. und 3 $^1/_4$ St. $3{,}000$.

Zusammenfassend kann gesagt werden: Innerhalb eines Wurzelpaares enthält jene Wurzel die geringste Anzahl Störungen, die in ihren Teilungen am weitesten gekommen ist. Eine ruhende Karyopse der Gerste enthält ausser der Hauptwurzel vier relativ grosse Wurzeln, die bei der Keimung in dem Masse mit Teilungen beginnen wie die Wasserzufuhr dies gestattet. Die in verschiedenem Zeitpunkt einsetzende Keimung der fünf Wurzeln bedeutet nicht, dass die Kerne sich in verschiedenem Abstand vom Reproduktionspunkte befinden sondern nur dass die Wasserzufuhr verschieden erfolgt. Bei der Bestrahlung lagen also die Ruhekerne in den verschiedenen Wurzeln in ungefähr gleichem Abstand vom Reproduktionspunkt. Aus diesem Grunde muss jene Wurzel, die mit den Teilungen am weitesten gekommen ist, eine geringere Anzahl Störungen aufweisen als jene, die gerade mit denselben begonnen hat.

Diese Erscheinung gilt hauptsächlich für die Trockenserien der Jahre 1934 und 1933. In den Serien von 1932 scheint ein Ausgleich stattzufinden, sodass die Wurzeln eine am ehesten zufallsmässige Verteilung der höchsten Störungsfrequenz aufweisen. In den Trocken- und 1 $^1/_2$ St.-Serien von 1931 zeigen jene Wurzeln, die in ihren Teilungen am weitesten gekommen sind, auch die grösste Anzahl Störungen (in sämtlichen drei Zusammenstellungen bestehen sichre Unterschiede). Ein Ausgleich scheint in den drei ersten Jahrgängen auch binnen den

nassbehandelten Serien stattzufinden: In 1) 42 : 28, 15 : 13, 11 : 10, in 2) 26 : 15, 10 : 9, 6 : 5 sowie in 3) 18 : 8, 7 : 4 und 2 : 1.

In den Serien von 1931 findet das gerade Entgegengesetzte statt. Während die Trocken- und 1 $^1/_2$ St.-Serien eine Korrelation zwischen hoher Anzahl Teilungen per Schnitt und hoher Störungsfrequenz aufweisen, ist das Verhältnis in den am stärksten wasserbehandelten Serien wieder das normale. Der Unterschied zwischen den zwei ersten und der letzten Serie ist trotz des unansehnlichen Materials sicher (χ^2=8,571, 7,021 und 3,000 in den drei Zusammenstellungen).

Die Wasserbehandlung der Karyopsen kommt, wie ich früher gezeigt habe, jenen Kernen zugute die zuerst zur Teilung schreiten. Die Untersuchungen über die relative Teilungsgeschwindigkeit machen klar, dass das in der Klasse 20—30 gelegene Minimum der Teilungsgeschwindigkeit in den 3 $^1/_4$ St.-Serien verschwunden und durch eine ungefähr konstante Teilungsgeschwindigkeit in sämtlichen Teilungsklassen ersetzt worden ist. Es hat also ein Ausgleich zwischen den Kernen stattgefunden. Die gleiche Erscheinung, obgleich in weniger extremen Grade, zeigt das Verhältnis Fragmentationen/Fusionen von 1934—1932 bei starker Wasserbehandlung. Die Anzahl Kerne mit einem hohen Wert für dieses Verhältnis nimmt in den Teilungsgebieten 0—40 (30) bedeutend zu. Der Ausgleich innerhalb der Wurzelpaare der Jahrgänge 1934—1932 bei Wasserbehandlung zeigt dasselbe.

Wie ist da der sichre Unterschied zwischen 1934—1932 und 1931 trocken und 1 $^1/_2$ St. zu erklären? — Mit dem Altern dürfte ein Prozess in die Karyopsen eingeführt worden sein, sodass die fünf in der Coleorrhiza gelegenen Wurzeln bei der Austrocknung 6. 10. 1934—24. 4. 1935 sich ungleich verhalten haben. Die Hauptwurzel beginnt am frühesten mit der Teilung (sie durchbricht zuerst die Fruchtwand und die Coleorrhiza), während die vier übrigen meistens zwei und zwei bei der Keimung in die Streckungs- und Teilungsphase eingehen. Die schwächsten Wurzeln verlieren wahrscheinlich nach einer gewissen Zeit von Altern am leichtesten das Wasser. Aber durch diesen Alterungsprozess bekommen die Chromosomen in diesen eine grössere Stabilität. Infolge der mit dem Altern parallelen Veränderung im Innern der Chromosomen und der Empfindlichkeit der Wurzeln für das Austrocknen erfolgt also mit dem Wasserverlust eine differentielle Stabilitätsänderung der Chromosomen. Zellkerne in den Wurzeln, die das Wasser nicht so schnell verloren haben, zeigen grössere Veränderung in den Chromosomen als die Kerne in den Zellpartien, wo der Wasserverlust am grössten gewesen ist.

Der Unterschied zwischen den Trocken- und den $1\,^1/_2$ St.-Serien von 1931 einerseits sowie der $3\,^1/_4$ St.-Serie von 1931 andererseits kann in Übereinstimmung mit obenstehendem Gedankengang auf einem Ausgleich zwischen den verschiedenen Wurzeln in einer Karyopse beruhen, nicht wie in früheren Jahrgängen auf einem Ausgleich zwischen den Kernen in der gleichen Wurzel. Die verschiedenen Wurzeln werden also untereinander gleichwertig ohne dass deshalb in gleicher Ausdehnung ein Ausgleich zwischen den Zellkernen stattfindet, welch letzteres darauf beruht, dass die zytologische Ungleichwertigkeit zwischen den Kernen in der Wurzel im Gegensatz zur Ungleichwertigkeit zwischen den Wurzeln allzu lange Zeit bestanden hat.

3. DIE ZYTOLOGISCHE NACHWIRKUNG.

Für die Serie, in der diese am deutlichsten beobachtet worden ist (1934 B III 23. 11), sind einige Zusammenstellungen (Tab. 6) gemacht worden, die mir in diesem Zusammenhang gewisse Bedeutung zu besitzen scheinen. Diese Serie zeigt nämlich nicht die grösste Menge zytologische Störungen im ersten Stadium des Keimungsprozesses (0—10 Teil./Schnitt), hier ist anstatt dessen der Prozent Störungen am geringsten. Werden die 11 untersuchten Wurzeln nach der Anzahl Teilungen per Schnitt geordnet, so ergibt sich folgende Serie: 0,75, 2,1, 2,4, 4,5, 12,6, 16,7, 18,1, 24,0, 31,6, 33,2, 40,7 mit folgenden entsprechenden Störungsfrequenzen: 0, 0, 42,9, 28,6, 47,8, 21,9, 22,1, 24,3, 41,0, 36,2 und 36 %. Die zwei Wurzeln mit der geringsten Anzahl Teilungen zeigen also keine Störungen, und die höchste Störungsfrequenz liegt im Gebiet 10—20 Teil./Schnitt.

In bezug auf die Anzahl von Metaphasen in den verschiedenen Klassen, zeigt das erste Gebiet ein entschiedenes Maximum (64,5 % gegenüber 58, 58, 57 und 54 % in den darauffolgenden). Der Unterschied zwischen 1934 B III 22/9 und 23/11 ist so gross, dass χ^2 5,148 erreicht, d. h. der Unterschied ist statistisch sicher.

Betrachtet im Lichte der darauffolgenden Untersuchungen über den differentiell tötenden Einfluss der Röntgenstrahlen werden diese Beobachtungen leicht erklärlich.

In einem ruhenden Samen gibt es nur Ruhekerne. Bei der Röntgenbestrahlung werden in den Chromosomen eine Menge von Störungen induziert. In dem Masse wie das Ruhestadium nach der Bestrahlung an Länge zunimmt, werden diese Störungen zunehmend stärker wirkend, aber gleichzeitig steigt auch der tötende Einfluss der Röntgenstrahlen, und es sterben gerade jene Kerne, die für die Bestrahlung am

empfindlichsten sind und am leichtesten Störungen erleiden, nämlich jene die zuerst in die Teilung eintreten sollten. Von den ersten Kernen in einer Wurzel werden also nur jene übrig bleiben, die aus dem einen oder anderen Grunde keiner so kräftigen Wirkung der Strahlen ausgesetzt worden sind. Daher die niedrige Störungsfrequenz im ersten Teilungsgebiet. Gleichzeitig nehmen diese ersten Kerne so stark an Anzahl im Verhältnis zu der darauffolgenden grossen Reihe von Kernen ab, dass das Maximum für die Teilungsgeschwindigkeit verschwindet.

TABELLE 6. *Die zytologische Nachwirkung (die 1934 B III 23/11-Serie).*

Die Prozentzahl von gestörten Zellen, Metaphasen und peripheren Teilungen in verschiedenen Teilungsgebieten.

	Teilungen per Schnitt				
	0 10	10 20	20 30	30 40	40 50
% Störungen	17,9 %	30,6 %	24,3 %	38,6 %	36 %
Anzahl der Wurzeln	(4)	(3)	(1)	(2)	(1)
% Metaphasen	$\frac{49}{76} = 64{,}5$ %	$\frac{200}{345} = 58{,}0$ %	$\frac{69}{120} = 57{,}5$ %	$\frac{257}{452} = 56{,}9$ %	$\frac{154}{285} = 54{,}0$ %
% periphere Teilungen	$\frac{15}{76} = 19{,}7$ %	$\frac{27}{101} = 26{,}7$ %	—	$\frac{47}{253} = 18{,}6$ %	—
Anzahl der Wurzeln	(4)	(1)	—	(1)	—

Die Verteilung der Teilungen in den Wurzeln bestätigen diese Schlusssätze. Während sämtliche übrigen Serien in den zwei äussersten Schnitten eine steigende Frequenz von peripheren Zellen mit einem Prozent unter 10 im ersten Gebiet (0—10) zeigen, hat diese Serie hier anstatt dessen fast 20 % periphere Teilungen und im nächsten Gebiet (10—20) 26,7 %. Das zweite Gebiet ist allerdings nur durch eine Wurzel repräsentiert, aber das erste durch vier Wurzeln. Auch diese Zahlen sprechen also dafür, dass die zentralen Kerne, d. h. die Kerne die dem Reproduktionspunkt am nächsten liegen, in grosser Ausdehnung getötet worden sind (siehe S. 28).

4. DIE VERÄNDERUNG DER MUTATIONSTYPEN MIT DEM ALTERN.

Obgleich die genetischen Resultate nach Röntgenbestrahlung in einem anderen Zusammenhang vorgelegt werden sollen, will ich hier im Zusammenhang mit dem zytologischen Mechanismus für das Altern einige genetische Resultate mitteilen. Die Versuche beziehen sich auf die sog. I-Serien (1937, S. 284).

Bei Röntgenbestrahlung von Gerste — in diesen Versuchen ausschliesslich Goldgerste — entstehen unter den Nachkommen der im Samenstadium bestrahlten Individuen eine Menge schon im Keimpflanzenstadium erkennbare genotypische Veränderungen, Chlorophyllmutanten. Diese Mutanten, die in den meisten Fällen subletal sind, bilden wegen ihrer Farbenabweichungen ein geeignetes Laboratoriummaterial, das es ermöglicht grosse Mengen von Individuen zu studieren. Die Farbenveränderungen sind mannigfaltig — gleichwie bei den spontanen Chlorophyllmutanten, siehe NILSSON-EHLE, 1922, und HALLQVIST, 1926 —: vollkommen weisse Typen (*albina*-Mutanten), gelbe mit verschiedenen Nuancen (*xantha*-Mutanten), gelbgrüne (*lutescens*-, *virescens*- und *chlorina*-Mutanten) sowie Typen mit Mischfarben. Von spontanen Mutanten mit Mischfarben ist die von NILSSON-EHLE (unveröff.) 1925 gefundene *alboxantha*-Mutante von besonderem Interesse. Die Blattspreite hat bei dieser eine klargelbe Farbe, die an der Spitze des Blattes durch einen weissgelben Farbenton ersetzt ist. Dieser Typus ist auch durch Bestrahlung erhalten worden. Andere Mutanten mit Mischfarben haben Blätter mit weisser Basis und gelber Spitze, weisser Basis und grüner Spitze, grüner Basis und weisser Spitze etc. Auch Mutanten mit abwechselnden Querstreifen von Grün und Gelb oder Braun und Gelb etc. (sog. Tigertypen) sind durch Bestrahlung erhalten worden. In sämtlichen Fällen ist eine vollkommene Dominanz für den Normaltypus vorhanden.

Bei diesen Untersuchungen sind in sämtlichen Serien Nachkommen jeder Pflanze für sich studiert worden. Von jeder Pflanze sind halbe Ähren ausgelegt worden und diese in der Ordnung, die durch die Länge des entsprechenden Halms bestimmt wird. Die Mutationsfrequenz wird aus der Anzahl *Pflanzennachkommen* mit Mutationen berechnet. Unter den Nachkommen derselben Pflanze tritt in seltenen Fällen mehr als eine Mutation auf. Die Mutation ist in der halben Ähre meistens durch ein Individuum, aber ausnahmsweise (siehe unten) durch zwei oder mehrere repräsentiert.

Da Samen mit hohem Wassergehalt nach der Bestrahlung durch-

weg eine höhere Frequenz zytologischer Störungen *unmittelbar nach Start der Keimung* aufweisen als Samen mit niedrigerem Wassergehalt, ist es von Bedeutung die sichtbare Mutationsfrequenz unter den Nachkommen festzustellen. Entstehen die Röntgenmutationen hauptsächlich durch Chromosomenstörungen der einen oder anderen Art, so soll der genetische Effekt parallel mit dem zytologischen gehen. In Tab. 7 sieht man, dass in den drei Jahrgängen, wo A- und B-Serien identisch bestrahlt worden sind, zwei (1934 und 1933) eine höhere Mutationsfrequenz in der A-Serie geben. Im übrigen Jahrgang (1931) geben die zwei Serien die gleiche Mutationsfrequenz. Es besteht ein schwacher Parallelismus.

TABELLE 7. *Die Veränderung der verschiedenen Mutationstypen nach Röntgenbestrahlung von gealterten Karyopsen.*

Serie	Anzahl der Mutationen	Absolute Anzahl der Mutationen	Anzahl der weissen Mutationen	Mutationen repräsentiert von mehr als einem Ind.		Σ Individ. von weissen Mutationen	Σ Individ. von nicht-weissen Mutationen
				Weisse Mutationen	Nicht-weisse Mutationen		
1934 A I	15,0 %	34	12 = 35,3 %	6 = 50,0 %	6 = 27,3 %	24(2,0)	30(1,4)
1934 B I	14,8 %	35	16 = 45,7 %	8 = 50,0 %	4 = 21,1 %	27(1,7)	23(1,2)
1933 A I	6,9 %	18	7 = 38,9 %	4 = 57,1 %	3 = 27,3 %	13(1,9)	14(1,3)
1933 B I	2,7 %	7	3 = 42,9 %	1 = 33,3 %	1 = 25,0 %	4(1,3)	5(1,25)
1932 A I	8,3 %	15	5 = 33,3 %	2 = 40,0 %	3 = 30,0 %	8(1,6)	14(1,4)
1932 B I	14,3 %	28	10 = 35,7 %	6 = 60,0 %	7 = 38,9 %	20(2,0)	31(1,7)
1931 A I	12,1 %	17	12 = 70,6 %	5 = 41,7 %	2 = 40,0 %	19(1,6)	9(1,8)
1931 B I	12,1 %	18	8 = 44,4 %	5 = 62,5 %	6 = 60,0 %	17(2,1)	19(1,9)
Σ		172	73 = 42,4 %	37 = 50,7 %	32 = 32,3 %	132(1,8)	145(1,5)

Die Frequenz von rein weissen Mutationen ist in den B-Serien ungefähr konstant (46, 43, 36 und 44 %). Hier entstehen also mit dem Altern keine Veränderungen. Die Frequenz von weissen Mutationen in den A-Serien liegen für 1934, 1933 und 1932 beträchtlich unter der Frequenz in den B-Serien. Aber in 1931 A I nehmen die *albina*-Mutationen stark an Anzahl zu (auf 71 %). Der Unterschied zwischen 1934 A I und 1931 A I ist statistisch sicher ($\chi^2 = 5{,}667$), desgleichen der Unterschied zwischen 1934, 1933 und 1932 A I einerseits und 1931 A I andererseits ($\chi^2 = 6{,}680$). Gleichwie die Chromosomen je nach dem Milieu in den Zellkernen eine verschiedene Sensibilität und Stabilität aufweisen, zeigen die Gene (oder gewisse Teile der Chromosomen) eine scheinbar

differentielle Stabilität in dem Masse wie die Physiologie der Zellkerne verändert wird. Und gerade die Samenserie, in der laut meiner Erklärung des Alterns die stärksten Veränderungen in den Zellkernen zu erwarten sind (1931 A I), zeigen diese Erscheinung in besonders hohem Grade.

Da die *albina*-Mutationen den sowohl spontan wie induziert häufigsten Mutationstypus darstellen, liegt die Annahme nahe zur Hand, dass sie in der einen oder anderen Weise chromosomal bedingt sind: die *albina*-Mutationen in der 1931 AI-Serie sollten an Anzahl zunehmen, weil die Chromosomenstörungen auffallend zahlreich geworden sind. — Dieser Schluss ist indessen fehlerhaft.

In Tabelle 7 ist auch die Häufigkeit der verschiedenen Mutationen angegeben. Die *albina*-Mutationen zeigen eine bedeutend grössere Individuenanzahl als die übrigen Typen. Dies ist umso bemerkenswerter als die *albina*-Mutationen phänotypisch extreme Typen darstellen. *Xantha*, *lutescens*, *virescens* etc. sind allerdings subletal, aber sie nähern sich doch dem normal grünen Zustand: Die *lutescens*- und *virescens*-Typen durch ein gewisses Vermögen zur Ausbildung von Chlorophyll, die *xantha*-Typen durch das Vermögen Xanthophyll auszubilden. Auch mit Hinsicht auf die Zerstörung der Chloroplasten bezeichnen die *albina*-Mutationen ein Extrem (EULER, BERGMAN, HELLSTRÖM und BURSTRÖM, 1936).

Von den insgesamt 73 *albina*-Mutationen treten nicht weniger als 37 in mehr als einem Individuum auf, von den übrigen anders beschaffenen 99 Mutationen treten nur 32 in mehr als einem Individuum auf. Der Unterschied zwischen den zwei Gruppen ist so gross dass $\chi^2 = 5{,}905$ erreicht. Der Unterschied zwischen weissen und nichtweissen Mutationen in dieser Hinsicht erreicht für die Serien von 1934 und 1933 einen noch grösseren Wert, nämlich $\chi^2 = 6{,}212$. Die oben genannten 37 weissen Mutationen kommen in 96 Individuen (2,6 Individuen per Mutation) vor, während die 32 nichtweissen Mutationen in 78 Individuen (2,4 Individuen per Mutation) auftreten.

Bei der Bombardierung von ruhenden Samen mit X-Strahlen entsteht eine Menge von sichtbaren Chromosomenstörungen, hauptsächlichst Fragmentationen, Fusionierungen und Translokationen. Je stärkere Veränderungen des Genotypus die induzierten Störungen bedingen (genisch und chromosomal), umso geringeren Selektionswert sollen sie besitzen. Am Weg hinauf bis zum Blütenstadium können daher nur solche Veränderungen bestehen bleiben, die den normalen Kernteilungsmechanismus nicht verhindern oder Störungen in der physiologischen

Tätigkeit des Kerns (Genotypus) mit sich bringen. Von den Mutationen, die bis hinauf zu den Reproduktionsorganen fortleben und deren Existenz erst in der nächsten Generation zutage tritt, bedeuten daher jene, die über die grössten Zellgebiete verbreitet sind, die geringsten Veränderungen des Genotypus. Da die *albina*-Mutationen unter den Nachkommen unbestreitbar mehr Individuen treffen als die übrigen Mutations-Typen, müssen sie daher in genetischer Hinsicht durchschnittlich weniger abweichen als letztere. Die starke Zunahme von *albina*-Typen in 1931 A I wird damit leicht erklärlich. Die Chromosomenstörungen sind so gewaltig (siehe S. 10) und von so wenig Selektionswert, dass nur die genetisch geringsten Störungen (Punktmutationen?) fortleben. Es ist da überraschend, dass die *albina*-Mutationen trotz ihres Charakters als kleine Mutationen in der A-Serie nicht in grösserer Individuenanzahl vorkommen als in der B-Serie. Die *albina*-Mutationen treten indessen hier in Zellorganen auf, die sich in einem abnormen Reizungszustand befinden. Sie sind daher, ohne selbst starke Veränderungen darzustellen, mit Störungen verbunden, die die normale Teilung der in Frage stehenden Kerne verhindern. In der 1931 B I-Serie, die nicht in gleicher Weise wie 1931 A I die abnorme Reizungsgrenze übersteigt, beträgt die Anzahl mutierter Individuen per *albina*-Mutation 2,1. Die entsprechende Zahl für 1931 A I ist 1,6. Auch 1932 B I verhält sich gleichartig: 2,0 mut. Individuen per *albina*-Mutation gegenüber 1,6 für 1932 A I.

Betrachten wir die Gesamtanzahl Mutationen mit mehr als einem mut. Individuum per Mutation in den verschiedenen Jahrgängen, so finden wir in den B-Serien 1934—1931 eine starke Zunahme von 34 und 29 % auf 46 und schliesslich 61 %, während die A-Serien ungefähr konstante Zahlen mit einer schwachen Zunahme für 1931 zeigen (35, 39, 33 und 41 %). Sowohl 1931 B I wie 1932 B I geben also mehr kleine Mutationen als 1931 A I und 1932 A I. Die Nass- und Trockenserien von 1934 und 1933 verhalten sich dagegen umgekehrt. Die Zahlen für die *albina*-Mutationen zeigen, wie oben erwähnt wurde, dasselbe. Die *albina*-Mutationen entstehen also nicht infolge der starken Chromosomenstörungen sondern trotz derselben.

Noch eine Erscheinung sei hervorgehoben. Auch die nichtweissen Mutationen, die mehr als ein Individuum betreffen, nehmen also mit dem Altern mehr und mehr an Anzahl zu. In 1934 und 1933 A I und B I sind sie noch ziemlich selten, sie betragen nur $^1/_4$ sämtlicher nichtweissen Mutationen. In 1932 A I und B I nimmt ihre Anzahl zu und noch ausgeprägter ist dies in 1931 A I und B I. In 1934 und 1933

liegen die A-Serien in dieser Hinsicht etwas über den B-Serien aber bei 1932 und noch ausgesprochener bei 1931 liegen die B-Serien über den A-Serien. In 1931 A I betragen die weissen Mutationen mit mehr als einem mut. Ind. 42 %, in 1931 B I 63 %, während die entsprechenden Zahlen für die nichtweissen Mutationen 40 % bzw. 60 % sind. Der Unterschied zwischen 1934—1932 A I und B I einerseits sowie 1931 A I und B I andererseits beträgt für diese nichtweissen Mutationen $\chi^2 = 3{,}563$, ist demnach nicht sicher. Der Unterschied zwischen 1934 A I und B I sowie 1931 A I und B I beträgt jedoch $\chi^2 = 4{,}222$, ist also signifikativ. Mit obenstehendem Gedankengang ist dies ganz natürlich. Auch von den vielen nichtweissen Mutationen werden 1932 und 1931 die grössten Abweichungen im Genotypus eliminiert (auch innerhalb der nichtweissen Mutanten bestehen natürlich Differenzen in dieser Hinsicht). In 1931 A I, die sich in einem abnormen Reizungszustand befindet, wird der Umfang dieser kleinen Mutationen zufolge eines unvermeidbaren Ballastes von minderwertigen zytologischen Störungen nicht so gross wie in der B-Serie.

Durch diese Untersuchung ist also festgestellt, dass die Nassserie von 1931 genetisch von den übrigen Serien abweicht.

III. DIE STERBLICHKEIT VON SAMEN UND ZELLKERNEN.

1. DIE DIFFERENTIELLE STERBLICHKEIT VON SAMEN MIT VERSCHIEDENEM WASSERGEHALT UND VON VERSCHIEDENEM ALTER.

Unbestrahltes Material. — Es ist eine in der Samenkontrolle und Praxis wohlbekannte Erscheinung, dass Samen mit hohem Wassergehalt in dem Masse wie sie altern eine niedrigere Keimkraft zeigen als Samen mit niedrigem Wassergehalt. Hier sei nur auf HEINRICH, 1913, verwiesen. Es kann von Bedeutung sein die Zahlen für die Sterblichkeit des von mir untersuchten Materials zu sehen (GUSTAFSSON, 1937, Tab. 2). χ^2 für den Unterschied zwischen 1934 A und B betrug am 17. 12. 1935 0,667, zwischen 1933 A und B 0,293, zwischen 1932 A und B 3,146 und zwischen 1931 A und B 2,917. Das verschiedene Verhalten zwischen 1934 und 1933 einerseits sowie 1932 und 1931 andererseits ist augenscheinlich. Werden 1934 und 1933 vereinigt, so erreicht χ^2 einen Wert von 0,829. Der entsprechende Wert für 1932 und 1931 beträgt 6,180, d. h. es besteht ein deutlicher Unterschied zwischen den zwei Gruppen von Jahrgängen. Ein erhöhter Wassergehalt bedingt eine Abnahme der Keimfähigkeit.

Bestrahltes Material. Verschiedener Wassergehalt (Tab. 8). — Zuerst die I-Serien. 1932 A I und B I wurden verschieden bestrahlt und müssen daher ausscheiden. χ^2 für den Unterschied zwischen den A- und B-Serien hat sowohl 1934 wie 1933 für das bestrahlte Material etwas zugenommen. χ^2 für den Unterschied zwischen 1931 A und B zeigt eine starke Zunahme von 2,917 auf 6,330.

TABELLE 8. *Die verschiedene Sterblichkeit der Karyopsen nach Röntgenbestrahlung.*

Serie	Anzahl der Karyopsen	Gekeimte Karyopsen	χ^2	Serie	Anzahl der Karyopsen	Gekeimte Karyopsen	χ^2
1934 A I	270	245	0,754	1934 A IV trocken, 1 St., 3 St.	589	514	0,870
1934 B I	277	257		1934 B IV trocken, 1 St, 3 St.	697	620	
1933 A I	283	257	0,319	1931 A IV trocken, 1 St., 3 St.	664	380	12,617
1933 B I	280	258		1931 B IV trocken, 1 St., 3 St.	498	336	
1931 A I	280	200	6,330	1934 A V	227	191	0,772
1931 B I	282	227		1934 B V	232	188	
				1935, 1934, 1931 A IV, B IV St.	962	231	0,194
				1935, 1934, 1931 A IV, B IV tr.	1057	245	
				1934, 1931 A VI, B VI 3 St.	915	590	1,524
				1934, 1931 A VI, B VI trocken	966	649	

Serie	Anzahl der Karyopsen	Gekeimte Karyopsen	%	Serie	Anzahl der Karyopsen	Gekeimte Karyopsen	%	Serie	Anzahl der Karyopsen	Gekeimte Karyopsen	%
1934 B III tr.	176	155	88,1	1934 B III 1 1/2 St.	141	117	83,0	1934 B III 3 1/4 St.	167	159	95,2
1933 B III tr.	214	172	80,4	1933 B III 1 1/2 St.	162	134	82,7	1933 B III 3 1/4 St.	171	141	82,5
1932 B III tr.	235	124	52,8	1932 B III 1 1/2 St.	190	146	76,8	1932 B III 3 1/4 St.	190	146	76,8
1931 B III tr.	112	14	12,5	1931 B III 1 1/2 St.	71	47	66,2	1931 B III 3 1/4 St.	86	63	73,3

In den IV-Serien erreicht χ^2 für den Unterschied zwischen einerseits 1934 A IV trocken, A IV 1 St., A IV 3 St. sowie andererseits 1934 B IV trocken, B IV 1 St., B IV 3 St. 0,870, während der Unterschied zwischen den entsprechenden Serien der 1931er Karyopsen auf $\chi^2 = 12{,}617$ steigt. In der V-Serie schliesslich zeigt die A V-Serie eine etwas höhere Keimfähigkeit als B V, aber ohne signifikativ zu sein.

Ein Vergleich zwischen den befeuchteten Serien einerseits sowie

den entsprechenden identisch bestrahlten Trockenserien andererseits (der IV- und VI-Serien) ergibt gleichfalls Unterschiede, wenngleich nicht signifikative. Der Unterschied zwischen den 1 St. befeuchteten 1935-, 1934-, 1931 A IV und B IV-Serien sowie den entsprechenden Trockenserien gibt für χ^2 0,191. Der entsprechende Unterschied zwischen 1934, 1931 A VI und B VI 3 St. sowie 1934, 1931 A VI und B VI trocken ergibt $\chi^2 = 1{,}524$. (Die Röntgenbehandlung der VI-Serien wird in einer späteren Abhandlung mitgeteilt.)

Zwischen den künstlich befeuchteten Serien sowie den entsprechenden Trockenserien besteht also ein schwacher, nicht sichrer Unterschied in der Empfindlichkeit für Bestrahlung. Zwischen den 1934 und 1933 A- und B-Serien besteht in meinem Material ein ähnlicher unsicherer Unterschied. Zwischen 1931 A und B entstehen dagegen starke Unterschiede. Es sei erwähnt, dass 1931 A und B vor der ersten Bestrahlung nur während ca. 6 Monaten, vor der vierten Bestrahlung 14 Monate verschieden verwahrt worden ist, während die zwei Serien vor der Separation während drei Jahren ganz gleich behandelt worden sind.

Verschiedenes Alter. — Die Tabellen zeigen die *gradweise erfolgende* Veränderung im Innern der Samen, die die zunehmende Sterblichkeit bedingt: 1934, 1933, 1932, 1931 B III trocken zeigen einen Keimungsprozent von 88,1, 80,4, 52,8 und 12,5 %. Die relativen Zahlen für das Keimungsvermögen in den B III Trockenserien sind 7,0, 6,4, 4,2 und 1, in den B III 1 $^1/_2$ St.-Serien 1,25, 1,25, 1,16 und 1, in den B III 3 $^1/_4$-Serien 1,29, 1,12, 1,05 sowie 1. Die entsprechenden Zahlen für das Verhältnis zwischen 1934 A IV und 1931 A IV sind in den Trockenserien 1,49, in den 1 St.-Serien 1,61, in den 3 St.-Serien 1,26, während die entsprechenden Zahlen für 1934 und 1931 B IV 1,36, 1,31 und 1,30 betragen.

Die Atmung eines Samens nimmt mit dem Wassergehalt stark zu und es besteht die Möglichkeit, dass die höhere Sterblichkeit und das schnellere Altern der Samen mit hohem Wassergehalt auf Störungen im Atmungsmechanismus *beruht.* In dieser Hinsicht werden namentlich HEINRICHS bekannte Untersuchungen als beweiskräftig betrachtet. Er konnte zeigen, dass Karyopsen, die ohne Luftwechsel verwahrt worden sind, die Keimfähigkeit bedeutend schneller verloren als Karyopsen, die bei beständiger Luftzirkulation verwahrt worden sind. Die Abnahme der Keimfähigkeit ist ausser vom Alter der Samenpartie von der Verwahrungstemperatur sowie vom Wassergehalt abhängig, wobei jedoch der Wassergehalt der ausschlaggebende Faktor ist (siehe auch LEHMANN und AICHELE, 1931, S. 234). Bei einem Wassergehalt von 4,5 % keimten

Karyopsen von Gerste bei 30° C zu 96 %, bei einem Wassergehalt von 10,8 % zu 63 % sowie bei einem Wassergehalt von 14 % zu 0 %, alles nach einem Zeitraum von 69 Wochen.

Beim Durchlüften sowohl mit gewöhnlicher Luft, gereinigter trockener Luft wie gewöhnlicher gereinigter Luft hält sich die Keimfähigkeit bei sowohl Roggen wie Gerste noch nach 70 Wochen unverändert. Eine schwache Abnahme bei Durchlüften mit feuchtigkeitsgesättigter Luft besteht bei beiden Getreidearten. Bei der Gerste ist die Keimfähigkeit auf 96 % gesunken, beim Roggen auf 91 %; beide Zahlen sind von der Keimfähigkeit nach 4 Wochen statistisch sicher getrennt (nach 2 Wochen ist die Keimfähigkeit etwas niedriger als nach 4, zweifellos abhängig von der Notwendigkeit einer Nachreife). χ^2 beträgt für Gerste 7,313, für Roggen 21,645. Die verminderte Sterblichkeit bei Luftzirkulation steht also mit dem normalen Verlauf der Atmung im Zusammenhang. Bei hohem Wassergehalt wird die Atmung jedoch so intensiv, dass auch bei Erneuerung der Luft die Entstehung von anaeroben Nebenprodukten in den ruhenden Samen nicht vermieden werden kann. Die Sterblichkeit steigt daher, obgleich bei weitem nicht so stark wie in Serien ohne Erneuerung der Luft.

Ist nun diese allmählich stattfindende Zerstörung des Atmungsmechanismus die *primäre* Ursache des Absterbens der Samen? — Beim Keimungsprozess ist das wichtigste Moment das Vermögen der Kerne zu Reproduktion, das Vermögen der Zellen zu Teilung. Würde die Zerstörung des Atmungsmechanismus an und für sich ein Abtöten dieses Vermögens bedeuten, dann müssten die gleichen Enzyme oder die gleichen chemischen Prozesse nicht nur bei der Atmung sondern auch bei der Reproduktion der Chromosomen oder beim Start der Kernteilung fungieren. Dies ist unwahrscheinlich. Die Zunahme der Sterblichkeit durch die Röntgenbestrahlung von feuchten Samen beruht daher primär nicht auf einer Zerstörung des Atmungsmechanismus, sondern auf einer direkten Zerstörung der Zellkerne.

Da indessen die Zerstörung des Atmungsmechanismus und die erhöhte Sterblichkeit parallel gehen, stehen die beiden Erscheinungen zweifellos im Zusammenhang. — Bei der Atmung von ruhenden Samen entstehen zufolge mangelhafter Sauerstoffzufuhr eine Menge von anaeroben Abbauprodukten mit Giftwirkung, z. B. Alkohol, ausserdem dürfte auch der Kohlensäuregehalt in den Zellen allmählich höchst wesentlich zunehmen. Hierdurch entsteht eine Vergiftung der ruhenden Kerne, die gleichbedeutend mit der Zerstörung sein kann, die (bei Röntgenbestrahlung und) beim Einfluss von Narkotika stattfindet.

Bei Luftzirkulation wird die Sauerstoffzufuhr erhöht und die schädlichen Nebenprodukte werden entfernt oder vollständiger verbrannt. Die Zerstörung des Atmungsmechanismus ist also eine sekundäre Erscheinung. Die primäre Ursache der Sterblichkeit ist die mit dem Alter sehr erhöhte Empfindlichkeit der Kerne und der Chromosomen (des Reproduktionsmechanismus).

2. DIE DIFFERENTIELLE STERBLICHKEIT DER ZELLKERNE.

Im Vorstehenden ist nachgewiesen worden dass jene Zellkerne zuerst altern, die mit der Teilung in einer Wurzel zuerst beginnen sowie dass Samen mit hohem Wassergehalt teils eine grössere Chromosomensensibilität (Labilität) aufweisen, teils bei hohem Alter genetische Abnormitäten sowie schliesslich niedrigere Keimfähigkeit (schnelleren Tod) zeigen. Als Erklärung gewisser Resultate mit Bezug auf die zytologische Nachwirkung wurde hervorgehoben, dass die startenden Zellkerne, die sonst infolge ihres hohen Wassergehaltes eine hohe Frequenz von zytologischen Abnormitäten zeigen, bei langer Verwahrung nach der Bestrahlung auch leichter getötet werden als die nachfolgenden. In meinen früheren Arbeiten habe ich den differentiellen Effekt auf verschiedene Kerne in der Weise erklärt, dass die Chromosomen in diesen Kernen zufolge des höheren Wassergehaltes sich auf die Mitose durch eine partielle Reproduktion vorbereitet haben und dass sie infolgedessen eine hohe relative Teilungsgeschwindigkeit zeigen und die Chromosomen eine verminderte Stabilität. Den endgültigen Beweis für die Richtigkeit dieser Erklärung glaube ich im Zusammenhang mit dem Nachweis des Verhaltens der eingeweichten Serien nach der Bestrahlung (1936, S. 501) erbracht zu haben. Die Erscheinung dass die ersten Zellkerne schneller altern und sterben, scheint eine neue und beweismögliche Erklärung für den Todesprozess und das Altern erbringen zu können.

Die longitudinale Sterblichkeit. — Unmittelbar beim Studium von bestrahlten Samen aus den 1931 Trocken- und 1 $^1/_2$ St.-Serien sowie von gewissen Samen aus den 1932 Trocken- und 1 $^1/_2$ St.-Serien wurde die hohe Sterblichkeit von Kernen und Zellen konstatiert. In der Nähe der Wurzelhaube jeder gekeimten Wurzel lag eine Partie mit geschrumpften Zellen, die häufig nicht imstande gewesen waren die Zellstreckung auszuführen und scharf von den oberhalb derselben gelegenen Teilen der Wurzel abwichen. Auch der Kerninhalt erwies sich durch sein abnormes Aussehen als tot.

Auf Grund der Bedeutung dieser Beobachtung wurde die Anzahl

Teilungen in gewissen Schnitten von Wurzeln der Samen der Jahrgänge 1934, 1932 und 1931 von sämtlichen drei Parallelserien ermittelt. Für 15 Wurzeln der 1934-Serien, 14 Wurzeln der 1932-Serien und 24 Wurzeln der 1931-Serien wurde die Verteilung der Zellteilungen in jenem Schnitt der Schnittserie bestimmt, der die meisten Teilungen enthielt. Von der Wurzelhaube gerechnet wurden die Wurzeln bei der verwendeten konstanten Vergrösserung (ca. 1000fach) in longitudinale Partien von Okularbreite eingeteilt. Zellteilungen gab es in 5—6 solchen longitudinalen Partien. Die wenigen in der Wurzelhaube vorhandenen Teilungen wurden zu Partie 1 gerechnet. Wie bei den übrigen Untersuchungen wurden nur Metaphasen-, Anaphasen- und Telophasenstadien gezählt. In den toten Zellenpartien gab es auch keine Pro-

TABELLE 9. *Die longitudinale Sterblichkeit der Nuclei.*

Serie	Prozentzahl der Teilungen in verschiedenen Bezirken						Anzahl der Teilungen	Anzahl der Schnitte	Teilungen per Schnitt
	I	II	III	IV	V	VI			
1934 B III tr. ...	18,7	24,4	22,2	18,6	10,2	6,0	216	5	43,2
1932 B III » ...	16,6	21,9	24,7	23,1	11,3	2,4	247	5	49,4
1931 B III » ...	6,6	34,3	34,7	21,0	3,3	—	181	6	30,2
1934 B III 1 1/2 St.	19,1	25,1	29,4	20,1	6,4	—	299	6	49,8
1932 B III »	22,7	21,9	30,5	18,6	5,2	1,1	269	7	38,4
1931 B III »	12,0	23,1	29,8	25,6	8,6	0,8	359	9	39,9
1934 B III 3 1/4 St.	26,1	31,4	32,9	6,4	3,2	—	188	4	47,0
1932 B III » ...	21,6	25,7	27,0	20,3	5,4	—	74	2	37,0
1931 B III » ...	20,4	27,2	24,7	21,4	6,0	0,2	401	9	44,6

phasen. Da die toten Partien geschrumpft und zusammengedrückt sind, fällt der Prozent Teilungen in den Zellenpartien 1 und 2 der partiell toten Wurzeln zu hoch aus.

Tabelle 9 zeigt die auffallenden Unterschiede zwischen den verschiedenen Gebieten hinsichtlich Teilungsfrequenz in Partie 1. In sämtlichen drei Parallelserien zeigt 1931 die niedrigste Prozentzahl (7, 12, 20 gegenüber 19, 19 und 26 % für 1934). 1932 nimmt eine Mittelstellung ein. Von den 1 1/2 St.-Serien liegt 1932 etwas über 1934. — Es ist demnach eine differentielle Sterblichkeit nachgewiesen. Von den 6 *longitudinalen* Partien der Teilungszone sterben jene am frühesten, die in der Nähe der dicken Wurzelhaube liegen.

Die zentrale laterale Sterblichkeit. — Im Kapitel über den Start der Mitose wurde nachgewiesen, dass die Kerne, die die Teilung in einer

Wurzel beginnen, durchweg in den zentralen Teilen liegen und dass der Prozent periphere Zellen einen verhältnismässig konstanten Wert in den Klassen 20—30—40—50 Teil./Schnitt erreicht. Später sinkt ihre Frequenz wiederum. Es zeigt sich indessen, dass die Anzahl in Teilung befindliche periphere Zellen in dem Masse zunimmt wie die Bestrahlungsintensität und die *Störungsfrequenz* steigt. Werden die 1934-Serien nach der Störungsfrequenz (wegen des in dieser Hinsicht unzulänglichen Materials musste 1934 B III 23. 11 ausgeschlossen werden; vgl. jedoch S. 17) geordnet, so ergibt sich folgende Serie: 1934 B V trocken: 28,4 % Stör. und 23 % periphere Teilungen, 1934 B III trocken: 14,2 % Stör. und 21,7 % periph. Teil., (1934 B III 1 $^1/_2$ St.: 12,1 % Stör. und 21,8 % periph. Teil., 1934 B IV 1 St.: 11,5 % Stör. und 20,1 % periph. Teil.), 1934 B IV trocken: 9,4 % Stör. und 19,6 % periph. Teil., (1934 B III 3 $^1/_2$ St.: 3,7 % Stör. und 18,6 % periph. Teil.). Die fallende Frequenz von peripheren Zellteilungen ist auffallend. Obgleich die eingeweichten Serien ohne weiteres in diese Serie hineinpassen, ist es vielleicht nicht berechtigt sie mitaufzunehmen, da die Wasserzufuhr die Physiologie der Samen in mancherlei Weise verändert. Es ist jedoch zu erwähnen, dass die Serien der Jahre 1932 und 1931 das gleiche Verhältnis in ihnen selbst aufweisen dürften, während die Trockenserie von 1933 dagegen zu wenig periphere Zellen im Verhältnis zu den eingeweichten Serien enthält.

χ^2 für den Unterschied zwischen 1934 B V und 1934 B III beträgt 1,646, zwischen 1934 B III und 1934 B IV trocken 3,478, sowie zwischen 1934 B V und 1934 B IV trocken schliesslich 9,449. (Die korrigierten Zahlen wurden benutzt, vgl. S. 32). Es ist also sicher, dass die stärkere Bestrahlung und die höhere Störungsfrequenz eine Zunahme der Anzahl peripherer Teilungen im Verhältnis zu den zentralen verursacht. Dies kann kaum in anderer Weise gedeutet werden, als dass die zentralen Ruhekerne leichter getötet werden als die peripheren; sie sind demnach bedeutend empfindlicher als diese. 1937, S. 309 wurde nachgewiesen, dass die Frequenz Störungen in den zentralen Teilen einer Wurzel erheblich gesteigert ist. Hier ist gezeigt worden, dass nicht nur die Störungsfrequenz sondern auch die Sterblichkeit höher ist. Die Zellhaufen die durchschnittlich den höchsten Wassergehalt aufweisen sind gleichzeitig die am leichtesten zu störenden und zu tötenden. Laut meiner Erklärung ist die Nähe zum Reproduktionspunkt das entscheidende Moment. Die Zellen, die die Mitose im Zentrum der Wurzeln starten, haben hohen Wassergehalt und befinden sich daher nahe dem definitiven Reproduktionspunkt. Aus diesem Grunde

zeigen sie einen höheren Prozent Störungen als die nachfolgenden Stadien, oder — wenn die einwirkenden Agentien hinlänglich stark geworden sind — sie werden leichter getötet.

Dass nicht nur die Störungsfrequenz sondern auch die Sterblichkeit in einem direkten Zusammenhang mit dem Wassergehalt steht, zeigt sich ausser durch vorstehende Diskussion auch durch das Verhalten der am stärksten befeuchteten Serien *in den startenden Stadien.* Tabelle 1 zeigt, dass diese sowohl in 1934 wie in 1933 3 $^1/_4$ St. in bedeutend grösserer Ausdehnung periphere Zellen enthalten als die entsprechenden Trocken- und 1 $^1/_2$ St.-Serien. Diese Unterschiede sind zu ausgeprägt um nur auf einem Zufall beruhen zu können. χ^2 beträgt für den Unterschied zwischen 1934 trocken und 3 $^1/_4$ St. 2,422, für 1934 1 $^1/_2$ St. und 3 $^1/_4$ St. 5,031 sowie für den Unterschied zwischen 1933 trocken und 3 $^1/_4$ St. 8,001. Trotzdem die am längsten eingeweichten Serien bedeutend weniger intensiv bestrahlt worden sind als die Trokken- und 1 $^1/_2$ St.-Serien, hat die erhöhte Wasserzufuhr in den 3 $^1/_4$ St.-Serien eine erheblich grössere Sterblichkeit mit sich gebracht, *und das erste Stadium im Keimungsprozess hat am meisten gelitten* (mit einem hohen Prozent von Teilungen im Okulargebiet III; siehe unten). Die Wasserzufuhr kommt den Kernen zugute, die zuerst mit der Teilung beginnen, diese werden m. a. W. in ihrer Teilungstätigkeit beschleunigt, sie zeigen eine höhere Frequenz von Störungen und die grösste Sterblichkeit.

Dass diese Abnahme in der Prozentzahl der zentralen Zellen nicht darauf beruht, dass die Wasserzufuhr das Vermögen der peripheren Zellen, mit der Teilung zu beginnen, erhöht, wird durch die Unterschiede in den 1934 3 $^1/_4$ St. 10—20- und 20—30-Gebieten sowie in den gleichen Gebieten von 1933 3 $^1/_4$ St. bewiesen. Diese beiden Unterschiede sind nämlich statistisch sicher; χ^2 beträgt 9,815 bzw. 5,133.

In 1931 3 $^1/_4$ St. liegt das Maximum von peripheren Teilungen nicht wie in 1934 und 1933 im Gebiet mit 10—20 Teil./Schnitt, sondern anstatt dessen im 30—40-Gebiet. Dies ist leicht zu verstehen. Sämtliche Kerne, die mit der Teilung beginnen, liegen zufolge des Alterns nahe dem Reproduktionspunkt. Die Wasserzufuhr begünstigt allerdings diese ersten Kerne, aber die stärkste Differenz zwischen zentralen und peripheren Teilen wird in jener Gruppe von Kernen entstehen, die am weitesten vom Reproduktionspunkt entfernt liegen.

Es ist von grösster Bedeutung festzustellen, dass noch lange bevor im Mikroskop eine longitudinale Veränderung der Kerne wahrgenommen werden kann, eine zentrale Sterblichkeit auftritt. Diese beginnt

also früher als die longitudinale. Dies stimmt gut mit der Erscheinung überein, dass die allerersten Zellen, die mit der Teilung beginnen, sich nicht in unmittelbarer Nähe der Wurzelhaube befinden sondern ein Stück weiter drinnen in den geschützteren Teilen. Darauf startet die Reihe von Teilungen in der Nähe der Wurzelhaube. Den Beweis für die früher eingetretene zentrale Sterblichkeit erbringt merkwürdigerweise die Tabelle über die longitudinale (Tab. 10). Bevor eine Sterblichkeit direkt beobachtet werden kann (wie in den Samen von 1931 und in geringerem Umfang in den von 1932), zeigen sich jedoch longitudinale Unterschiede (in 1934). Die negativen Differenzen zwischen 1934 trocken und 1 1/2 St. bzw. 3 1/4 St. in den sechs verschiedenen Okulargebieten sind im Gebiet III am grössten, nämlich 7 bzw. 11 %, d. h. die Sterblichkeit ist hier am stärksten zutage tretend. χ^2 beträgt hierfür 3,353 und 5,868, während χ^2 für

TABELLE 10. *Die Differenzen zwischen den Trocken-, resp. 1 1/2 St.- und den 3 1/4 St.-Serien (D_1 und D_2) in verschiedenen longitudinalen Bezirken.*

Serie	I	χ^2	II	χ^2	III	χ^2	IV	V	VI
1934 B III	D_1 — 0,4 %	—	— 0,7 %	—	— 7,2 %	3,353	— 1,5 %	+ 3,8 %	+ 6,0 %
	D_2 — 7,4 %	3,329	— 7,0 %	2,350	—10,7 %	5,868	+12,2 %	+ 7,0 %	+ 6,0 %
1932 B III	D_1 — 6,1 %	3,002	0,0 %	—	— 5,8 %	2,152	+ 4,5 %	+ 6,1 %	+ 1,3 %
	D_2 — 5,0 %	—	— 3,8 %	—	— 2,3 %	—	+ 2,8 %	+ 5,9 %	+ 2,4 %
1931 B III	D_1 — 5,4 %	3,767	+11,2 %	—	+ 4,9 %	—	+ 4,6 %	— 5,3 %	— 0,8 %
	D_2 —13,8 %	17,578	+ 7,1 %	—	+10,0 %	—	— 0,4 %	— 2,7 %	— 0,2 %

den Unterschied zwischen 1934 trocken I und 1934 3 1/4 St. I 3,329 erreicht und zwischen 1934 trocken II und 1934 3 1/4 St. II 2,350. Dieser sichere Unterschied im Gebiet III wird ohne Schwierigkeit durch die zentrale Sterblichkeit erklärt: Gerade im Gebiet III befinden sich jene Teilungen, die zuerst zu beobachten sind. In 1932 ist dieser Unterschied nicht so ausgeprägt: χ^2 beträgt hier für den Unterschied zwischen 1932 trocken und 1 1/2 St. im Gebiet III nur 2,152, während es im Gebiet I 3,002 erreicht. In 1931 schliesslich sind die Differenzen im Gebiet I die einzigen beobachtbaren: χ^2 wird hier 3,767 bzw. 17,578. Beim Altern wird demnach die zentrale Wirkung verschoben. Dies bedeutet nicht, dass die Zellen im Gebiet III *nicht* getötet worden sind, sondern dass die hier getöteten Zellen in geringerer *Anzahl* vorkommen als die getöteten Zellen im Gebiet I. Die Physiologie der startenden Zellkerne zeigt, dass diese Kerne nur einen geringen Teil sämtlicher teilungsfähigen Kerne

TABELLE 11. *Die Sterblichkeit der zentralen Nuclei.*

Serie	Anzahl der peripheren Teil.	χ^2	Serie	Anzahl der peripheren Teil.	χ^2	Serie	Anzahl der peripheren Teil.	χ^2
Unkorr.		a—d	Unkorr.		a—d	Unkorr.		a—d
1934 B III tr.	$\frac{713}{3290} = 21{,}7$ %	—	1934 B III 1 1/2 St.	$\frac{1100}{5041} = 21{,}8$ %	0,314	1934 B III 3 1/4 St.	$\frac{844}{4531} = 18{,}6$ %	16,192
		b—d			b—d			b—d
1933 B III »	$\frac{296}{1553} = 19{,}1$ %	6,162	1933 B III »	$\frac{300}{1459} = 20{,}6$ %	—	1933 B III »	$\frac{357}{1597} = 22{,}4$ %	—
		c—d			c—d			c—d
1932 B III »	$\frac{413}{1891} = 21{,}8$ %	—	1932 B III »	$\frac{194}{1023} = 19{,}0$ %	1,990	1932 B III »	$\frac{233}{1075} = 21{,}7$ %	—
1931 B III »	$\frac{141}{590} = 23{,}9$ %		1931 B III »	$\frac{381}{1798} = 21{,}2$ %		1931 B III »	$\frac{481}{2103} = 22{,}9$ %	
			Unkorr.		a—d			
			Σ 1934 B III.....	$\frac{2657}{12862} = 20{,}7$ %	5,616			
					b—d			
			Σ 1933 B III.....	$\frac{953}{4609} = 20{,}7$ %	3,699			
					c—d			
			Σ 1932 B III.....	$\frac{840}{3989} = 21{,}0$ %	2,020			
			Σ 1931 B III.....	$\frac{1003}{4491} = 22{,}3$ %				
Korr.		a—d	Korr.		a—d	Korr.		a—d
1934 B III tr.	$\frac{713}{3286} = 21{,}7$ %	3,583	1934 B III 1 1/2 St.	$\frac{1015}{4582} = 22{,}2$ %	0,402	1934 B III 3 1/4 St.	$\frac{801}{4380} = 18{,}3$ %	18,819
		b—d			b—d			b—d
1933 B III »	$\frac{250}{1285} = 19{,}5$ %	7,847	1933 B III »	$\frac{300}{1459} = 20{,}6$ %	—	1933 B III »	$\frac{313}{1445} = 21{,}7$ %	—
		c—d			c—d			c—d
1932 B III »	$\frac{405}{1813} = 22{,}3$ %	2,095	1932 B III »	$\frac{174}{910} = 19{,}1$ %	1,822	1932 B III »	$\frac{233}{1075} = 21{,}7$ %	—
1931 B III »	$\frac{137}{541} = 25{,}3$ %		1931 B III »	$\frac{338}{1580} = 21{,}4$ %		1931 B III »	$\frac{481}{2103} = 22{,}9$ %	
			Korr.		a—d			
			Σ 1934 B III.....	$\frac{2529}{12248} = 20{,}6$ %	7,408			
					b—d			
			Σ 1933 B III.....	$\frac{863}{4189} = 20{,}6$ %	5,118			
					c—d			
			Σ 1932 B III.....	$\frac{812}{3798} = 21{,}4$ %	1,826			
			Σ 1931 B III.....	$\frac{956}{4224} = 22{,}6$ %				

ausmachen. Die sehr empfindlichen Kerne im Gebiet III sind in bedeutend geringerer Anzahl vorhanden als die etwas weniger empfindlichen Kerne in den Gebieten I und II. Wenn der Einfluss der einwirkenden Agentien genügend stark wird, wird die differentielle zentrale Sterblichkeit vom Gebiet III nach den Gebieten II und I verschoben, d. h. das Verhältnis tritt auf, das man gerade in den Samen der Jahrgänge 1932 und 1931 vorfindet.

Da das Okulargebiet III von mehreren Gesichtspunkten wichtig ist, sind die absoluten Zahlen für seine Ausdehnung in der Wurzel von einem gewissen Wert. Jedes Okulargebiet umfasst eine Länge von 0,14—0,15 mm. Die teilungsfähige Zone umfasst in *diesen Untersuchungen* über die *erste* Teilung der Zellen ungefähr 0,9 mm. Das Gebiet Nummer III liegt also zwischen 0,30—0,45 mm von der äussersten Spitze der Wurzel (die Wurzelhaube nicht mitgerechnet), d. h. etwas unter der Mitte der teilungsfähigen Zone.

Im Vorstehenden ist nur die differentielle zentrale Sterblichkeit innerhalb der gleichen Jahresklasse behandelt worden. Es verbleibt nun über die zentrale Sterblichkeit im Zusammenhang mit dem Altern zu berichten (Tabelle 11). Hier findet man in der ersten und zweiten Abteilung die Zahlen nach Vereinigung sämtlicher Wurzeln, also auch der Werte aus den Klassen 0—20 Teil./Schnitt (siehe S. 1), die ausser in den 3 1/4 St. eingeweichteten Karyopsen sich durch einen niedrigen Prozent peripherer Zellen auszeichnen. Die letztgenannten Serien enthalten zufolge früher klargelegter Gründe einen Überschuss an peripheren Zellen. Diese abweichenden Werte, allzu niedrig oder allzu hoch, sind in der dritten und vierten Abteilung ausgeschlossen. Hier sind also die korrigierten und die aus diesem Grunde sichersten Werte mitgeteilt.

Die Unterschiede (χ^2) sind zwischen den drei Jahrgängen 1934—1932 einerseits und 1931 andererseits berechnet worden. Die wertvollsten Zahlen sind natürlich die der Trockenserie, da die Wasserzufuhr in den anderen Serien gewisse physiologische Komplikationen verursacht. In der Trockenserie zeigt 1931 wie erwartet die höchste Anzahl periphere Zellen (24 % gegenüber 22, 19 und 22 %; der Unterschied zwischen 1933 und 1931 ist sicher). Der älteste Jahrgang zeigt somit eine grössere Frequenz peripherer Zellen als die drei anderen Jahrgänge und damit auch eine höhere zentrale Sterblichkeit. Noch besser geht dies aus den korrigierten Werten hervor. Für diese betragen die drei Werte für χ^2 3,533, 7,847 und 2,095.

Auch die 3 1/4 St. eingeweichten Serien machen dies klar. Hier

beträgt χ^2 für den Unterschied zwischen 1934 und 1931 den grossen Wert 16,192. 1933 und 1932 zeigen auch niedrigere Prozentzahlen für die peripheren Zellen als 1931, aber diese sind ohne Sicherheit. Die korrigierten Zahlen ergeben für den Unterschied 1934—1931 $\chi^2 = 18{,}819$.

Die 1 1/2 St. eingeweichten Serien weichen ab. Hier sind so gut wie keine Unterschiede vorhanden. Der grösste Wert für χ^2 der korrigierten Zahlen (zwischen 1932 und 1931) geht allerdings in die richtige Richtung, beträgt aber nur 1,990, während das einzige χ^2 in die falsche Richtung (zwischen 1934 und 1931) nur 0,314 ausmacht. Es ist offenbar, dass die Trockenserien zufolge der starken Bestrahlung die stärksten Ausschläge geben. In den 1 1/2 St.-Serien ist die Wasserzufuhr nicht gross genug gewesen um die verminderte Bestrahlungsintensität aufzuwiegen, was dagegen in den 3 1/4 St.-Serien der Fall gewesen ist.

Werden sämtliche drei Parallelserien für die vier Jahresklassen vereinigt, so resultieren für χ^2 der unkorrigierten Zahlen 5,616, 3,699 sowie 2,020 und für die korrigierten Zahlen 7,408, 5,118 und 1,826, d. h. fallende Werte. Zwischen 1934 und 1933 einerseits sowie 1932 und 1931 anderseits erreicht χ^2 3,958 für die unkorrigierten Zahlen und 6,375 für die korrigierten Zahlen, d. h. die Unterschiede sind sicher.

Die im Zentrum grössere Sterblichkeit dürfte damit ebenso wie die longitudinale Sterblichkeit bewiesen sein.

IV. DER TOD UND DER REPRODUKTIONSMECHANISMUS DER CHROMOSOMEN.

Aus den in vorliegender und den beiden früheren Arbeiten mitgeteilten Tatsachen geht deutlich hervor, dass die Störungsfrequenz und die Sterblichkeit nach Röntgenbestrahlung in Kernen mit hohem Wassergehalt am grössten ist. Dass dies nicht auf einem physikalischen Prozess beruht geht deutlich aus meiner Arbeit von 1936 hervor. Die Fusionsfrequenz ist eine Funktion der Stärke der Röntgenbestrahlung, ist aber anstatt erhöht zu sein in den frühesten Stadien der dort analysierten Serien vermindert. Nur in den stark gealterten Serien 1931 B III 1 1/2—3 1/4 St. zeigt die Fusionsfrequenz eine starke Erhöhung in den ersten in Teilung begriffenen Kernen (S. 10). Auch die auf S. 11 besprochene Untersuchung zeigt, dass in in natürlicher Weise gealterten Samen *die ersten Zellteilungen* am stärksten gealtert und daher abnorm sind. Der differentielle Effekt der Röntgenbestrahlung entschleiert also eine mit dem Altern erfolgende biologische Veränderung in den Kernen und Chromosomen.

Die Lebensprozesse in einer Zelle sind bei hohem Wassergehalt intensiver als bei niedrigem. Darauf beruht die grössere Sterblichkeit von Samen mit hohem Wassergehalt und das differentielle Altern von Zellen in einem ruhenden Samen. — Die für das Leben einzige Möglichkeit zu seiner Erhaltung ist sein Vermögen zu Reproduktion. Diese ist an den Zellkern gebunden, näher bestimmt an die Gene (die Chromosomen). Die Reproduktion der Gene repräsentiert eine Mannigfaltigkeit verwickelter chemischer Prozesse, deren Intensität gleichwie die der übrigen Lebensprozesse mit dem Wassergehalt in den Kernen zunimmt. Erst bei einem gewissen Minimiwert für den Wassergehalt kann die Reproduktion der Chromosomen endgültig stattfinden und diese verursacht damit den Start der Mitose. In Samen mit hohem Wassergehalt, wo dieser jedoch für die Keimung (die Reproduktion) zu niedrig ist, verlaufen Prozesse im Innern der Chromosomen, die ihre Stabilität vermindern. Gleichzeitig wie der Wassergehalt in den Samen weiter vermindert wird, nehmen die in den Kernen verlaufenden Prozesse an Stärke ab, wodurch die Stabilität der Chromosomen erhöht wird.

In dem Masse wie die Samen altern, werden die Äusserungen der scheinbar unbefindlichen Lebensprozesse akkumuliert und die Störungen in ihrem normalen Verlauf erhöht. In Kernen mit hohem Wassergehalt sind die Störungen am stärksten. Die verwickelten chemischen Prozesse, wodurch die Gene und das akzessorische Material in den Chromosomen reproduziert werden, werden gleichwie die übrigen Lebensprozesse mit dem Altern verändert. Auch in Kernen mit niedrigem Wassergehalt erfolgen Veränderungen im Innern der Gene und Fibrillen. Die Tausende von Elementen in den Chromosomen sind nicht ganz leblos sondern jedes von ihnen versucht seine Reproduktion zu vollziehen, wodurch die Störungen allmählich stärker und stärker werden. Schliesslich ist der in Frage stehende Kern lebensunfähig und stirbt oder, wenn er während den Todeszuckungen durch Wasserzufuhr überhaupt zur Teilung gebracht werden kann, zeigt er starke Veränderungen in den Chromosomen, niedrige Teilungsgeschwindigkeit u. s. w. Die trockensten Kerne zeigen also das schwächste Zeichen für Leben, halten sich dadurch aber auch länger am Leben. Der Tod wird allmählich durch eine zu starke Aktivität im Kern während den im ruhenden Samen ungünstigen Umständen verursacht. (Die allmähliche Stabilitätsverminderung der Chromosomen beim Altern wird somit durch Änderungen im Reproduktionsmechanismus verursacht, diese sind aber ausser vom Wassergehalt auch von anderen Faktoren mit beeinflusst.)

Laut dieser Hypothese für das Altern und den Tod entstehen diese negativen Lebensäusserungen in dem Masse wie die Ruhekerne an ihrer Teilung gehindert werden. Die Ursachen für das Altern in einem Zellkörper sind also in den Faktoren zu suchen, die die vollständige Reproduktion der Gene (der Chromosomen) und die Durchführung der Kernteilung verhindern. In dieser und in vorhergehenden Arbeiten ist *ein* derartiger Faktor nachgewiesen worden, nämlich der Wassermangel.

SUMMARY.

Title of paper: *Death as a nuclear process.* The paper reports further results from the investigation on X-raying of barley seeds, some results of which, together with a description of material and methods, were published in GUSTAFSSON, 1936 and 1937.

1. Ageing does not cause a discontinuous increase in the sensitivity of the chromosomes to X-rays. Instead, the properties of the chromosomes are gradually changed.

2. In the progeny of plants, derived from the oldest X-rayed seeds with a high water content, the frequency of *albina* mutations in proportion to the frequency of other chlorophyll mutations is much higher than in the other groups of material. — Presumably the *albina* mutations are on an average associated with less chromosome disturbances than the other mutations and thus cells and cell-groups with the *albina* mutation have a greater chance of propagating within the plant and partaking in embryo formation.

3. The ageing process, ending in the loss of germination power, proceeds faster in seeds with a high water content than in seeds with a low water content.

4. The nuclei in a root tip, which have the highest water content, are the first to start division, and are thus nearest to the reproduction point. These nuclei are most sensitive to exterior agencies, showing the highest frequency of chromosome disturbances and being killed by a comparatively small dosage of X-raying.

5. Even in seeds, which are not exposed to exterior agencies, these nuclei show the greatest amount of chromosome disturbances (cf. NAVASHIN and GERASSIMOVA, 1935).

6. The increase in mortality, caused by increased dosage of X-rays, is more rapid in the central nuclei of a root than in the peripheral ones.

7. In seeds, moistened for $3\,^1/_4$ h., the central nuclei of roots just

beginning to germinate show the highest rate of mortality upon X-raying.

8. Also in the ageing of seeds the sensitivity and mortality of central nuclei are greatly increased.

9. After intense X-raying of aged seeds groups of cells in the vicinity of the root cap are killed. Owing to the great number of these dead nuclei their unfitness for life is directly visible in the microscope.

10. The part of a root, which contains the nuclei most sensitive to exterior agencies and most easily killed, is situated between 0,30 and 0,45 mm. above the extreme point of the root (the root cap excluded), i. e. somewhat below the middle of the zone of dividing cells.

11. The results summarized above show that the greatest sensitivity to exterior agencies and the highest rate of mortality is found in those cells, which are the first to divide, when germination sets in, and which also have the highest water content (cf. GUSTAFSSON, 1936 and 1937). The presumed explanation of this correlation between rapid division and mortality implies a special hypothesis as regards the causes of the manifestations of life in the »resting» seed, which are called ageing and death. — The nuclei of the embryo in the seed are not really resting, but vital processes are slowly going on, preparatory to the reproduction of chromosomes and nuclei. If these processes have advanced to a certain stage but external conditions prevent their fulfilment in the actual division, the nuclei degenerate and die. The reproduction of the chromosomes implies a great number of intricate chemical processes, and it seems probable that the rate of these processes, as well as that of many other vital processes, is enhanced by an increase in the water content of the cells. Thus, cells with a high water content should be the first to reach the stage, where division sets in normally. If, however, the water content is insufficient for the division, these cells are also the first to begin to degenerate, and will be the first to die.

Ich bin Herrn Dr. O. TEDIN, Svalöf, für viele Diskussionen, für wertvolle Ratschläge und für die Zusammenfassung in englischer Sprache vielen Dank schuldig.

Svalöf, den 15. September 1936.

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INHALT.

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LENGTH FACTORS IN DWARF PEAS

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INTRODUCTION.

THE distinction between tall and dwarf peas has been known to be due to a single factor difference since the days of MENDEL. More recently, however, it has been shown that other factors affect the expression of MENDEL's recessive dwarf type. RASMUSSON (1927) obtained a type, which he called crypto-dwarf, in $^1/_{16}$ of the F_2 progeny of a cross between two dwarfs. Also DE HAAN (1927), on crossing two dwarfs, found an F_2 segregation of 15 : 1 for normal dwarfs and a new type called slender. Complete descriptions of the crypto-dwarf and slender types are given by these authors. In a later paper (1930) DE HAAN gives data concerning the relation between RASMUSSON's crypto-dwarf type and his own slender. He crossed a dwarf type, recessive for one of the slender factors, with crypto-dwarf and obtained a 3 : 1 segregation, based on 214 plants, for dwarf: crypto-dwarf in the F_2. A second cross between crypto-dwarf and slender gave an F_2 of 854 plants segregating in a proportion of 3 crypto-dwarfs and 1 slender. From this he appears to have drawn the conclusion that one of the two crypto-dwarf factors is identical with one of the slender factors but that the other factor is distinct from that of slender. However, it will be noticed that crypto-dwarf $\times$ dwarf gives an F_2 of 3 dwarfs : 1 crypto-dwarf, slender $\times$ the same dwarf gives an F_2 of 3 dwarfs : 1 slender, while finally crypto-dwarf $\times$ slender F_2 has 3 crypto-dwarfs : 1 slender.

This furnishes very strong evidence that the second crypto-dwarf factor and the second slender factor are multiple allelomorphs. It was decided to test this hypothesis further, particularly as DE HAAN states that his work on the relation between crypto-dwarf and slender was only of a preliminary nature.

MATERIAL AND METHODS.

11 pure breeding strains were chosen as material for the investigation. Of these, 7 were dwarf, 2 crypto-dwarf and 2 slender. I am indebted to Dr. J. RASMUSSON and Mr. ERNST NILSSON for supplying

these plants. Table 1 gives the name and source of each of these lines together with the symbols adopted for them in this paper.

TABLE 1. *No., Name, Origin and Symbols of Basic Material.*

No.	Name	Supplied by	Symbol
1	Frühe niedrige	NILSSON	*Fr*
2	Grey dwarf	RASMUSSON	*Gd*
3	201 . 1	DE HAAN	*201 . 1*
4	Witham wonder	RASMUSSON	*Ww*
5	English wonder mutant	NILSSON	*Engl. w*
6	204 . 1	DE HAAN	*204 . 1*
7	Acacia	NILSSON	*Ac*
8	Crypto-dwarf lines	RASMUSSON	Cdw_R
9	» » »	NILSSON	Cdw_N
10	Slender line	DE HAAN	Sl_{dH}
11	» »	NILSSON	Sl_N

Diallel crosses were made between all of these varieties as far as possible. In Table 2 the crosses are numbered from 1—55. Crosses not performed are placed within brackets.

TABLE 2. *Crossing scheme. Crosses not performed are placed within brackets.*

Gd	*201 . 1*	*Ww*	*Engl. w*	*204 . 1*	*Ac*	Cdw_R	Cdw_N	Sl_{dH}	Sl_N	Parents
1	2	3	4	5	6	7	8	9	10	*Fr*
	11	12	(13)	14	15	(16)	(17)	18	19	*Gd*
		20	(21)	22	23	24	25	26	27	*201 . 1*
			(28)	29	30	(31)	32	33	34	*Ww*
				(35)	(36)	(37)	(38)	(39)	(40)	*Engl. w*
					41	42	43	44	45	*204 . 1*
						46	47	48	49	*Ac*
							50	51	52	Cdw_R
								53	54	Cdw_N
									55	Sl_{dH}

Of each combination different parental plants were crossed. In the present paper a »family» represents the progeny of a single pair of parents. (It is thus possible to investigate if the parental lines are pure.)

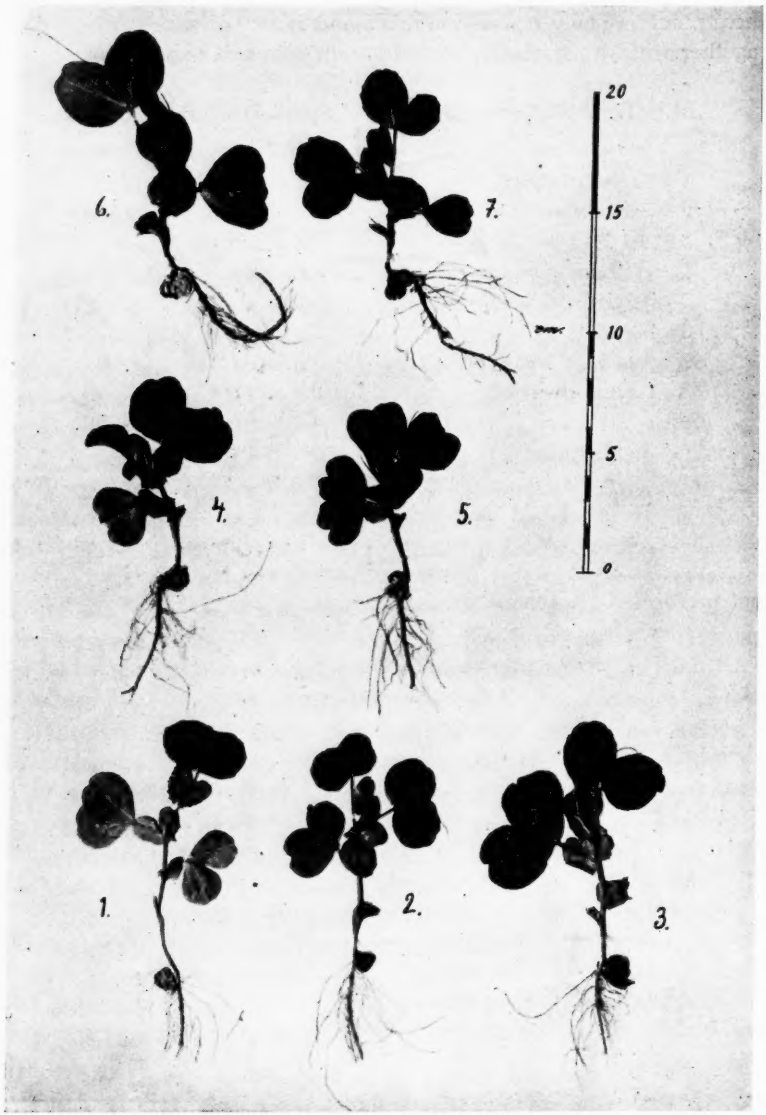

Fig. 1. Internode length of juvenile dwarf plants of genically different types. — *1*: Frühe niedrige ($cy_1cy_1Cy_2Cy_2$). — 2: Grey dwarf ($cy_1cy_1Cy_2Cy_2$). — *3:* 201 . 1 ($cy_1cy_1Cy_2Cy_2$). — *4*: Witham wonder ($Cy_1Cy_1cy_2^{c}cy_2^{c}$). — *5*: English wonder mutant ($Cy_1Cy_1cy_2^{c}cy_2^{c}$). — *6*: 204 . 1 ($Cy_1Cy_1cy_2^{s}cy_2^{s}$). — 7: Acacia ($Cy_1Cy_1cy_2^{s}cy_2^{s}$). — Note that the dwarfs Nos. 1—3 have slightly longer internodes than the dwarfs Nos. 4—6.

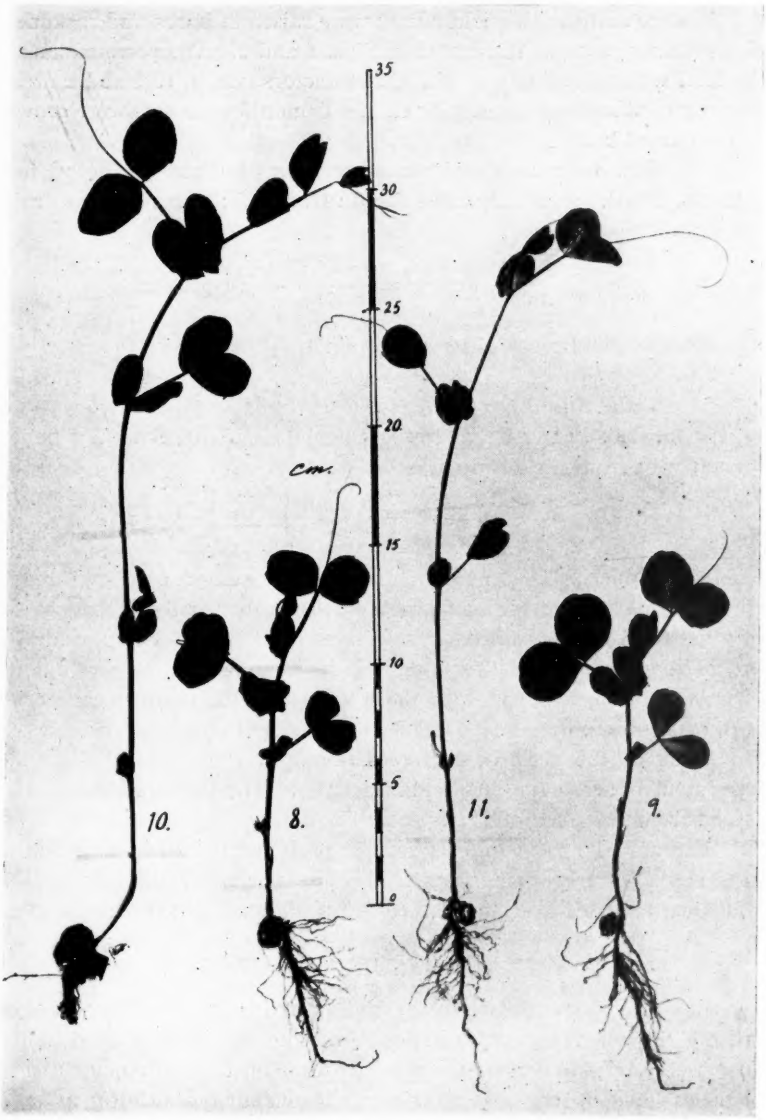

Fig. 2. Internode length of juvenile crypto-dwarf and slender plants of genically different types. — *8*: Crypto-dwarf-Rasmusson ($cy_1cy_1cy_2^ccy_2^c$). — *9*: Crypto-dwarf-Nilsson ($cy_1cy_1cy_2^ccy_2^c$). — *10*: Slender-De Haan ($cy_1cy_1cy_2^scy_2^s$). — *11*: Slender-Nilsson ($cy_1cy_1cy_2^scy_2^s$). — All the plants in Figs. 1 and 2 are of the same age.

F_1 were sown in the field but F_2 were raised in boxes and reckoned in the young stage, as illustrated in Figs. 1 and 2. The germination of the seed was exceedingly good. Other factors, viz. *r*, *tl*, *i* and *a* were also recorded in some cases. F_3 families from a few crosses were grown as mentioned below.

The data were analysed statistically by the use of the χ^2 test (FISHER, 1936). χ^2 is calculated for 3 : 1 and 15 : 1 segregations from the simplified formulae:

$$\chi^2 = \frac{(a - 3b)^2}{3n} \text{ and } \chi^2 = \frac{(a - 15b)^2}{15n} \text{ respectively.}$$

a = the number of observed dominant plants,
b = » » » » recessive »
n = » total number of observed plants.

χ^2 for 12 : 3 : 1 segregations is calculated in the generally better known way from the formula

$$\chi^2 = \sum\left(\frac{(a - m)^2}{m}\right)$$

where a is the observed number and m the expected number in each class, summation proceeding over all classes in the family. The analysis of χ^2 is based on that described by FISHER and is rendered possible by the additive property of this quantity. In the analysis, the »deviation» χ^2 tests the agreement between the totals of all the families concerned with expectation, the »reciprocal differences» χ^2 the agreement between the totals of the reciprocal crosses, and the »heterogeneity» χ^2 the agreement between families within a cross. The probabilities are obtained from the table of χ^2 given by FISHER.

The analysis of χ^2 into its various parts, each testing a particular agreement, clearly demonstrates the superiority of this method of statistical analysis over the use of the traditional D/m method.

RESULTS.

In order to coordinate the symbols for this group of factors they will all be called »*cy*» with indices. On the theory developed in the first section crypto-dwarfs should have the constitution $cy_1cy_1cy_2{}^c cy_2{}^c$, slenders $cy_1cy_1cy_2{}^s cy_2{}^s$ and dwarfs may be of four types $Cy_1Cy_1cy_2{}^c cy_2{}^c$, $Cy_1Cy_1cy_2{}^s cy_2{}^s$, $cy_1cy_1Cy_2Cy_2$ and $Cy_1Cy_1Cy_2Cy_2$ where cy_1 is the recessive factor common to crypto-dwarf and slender, $cy_2{}^c$ the second recessive crypto-dwarf factor and $cy_2{}^s$ the second recessive slender factor. (Note:

TABLE 3. *F_2 analysis.*

Cross No	Parents	Number of families tested	Observed number of			Total number of plants	χ^2 for			Probability for		
			Dwarf	Cdw	Slender		Deviation from expectation	Difference between reciprocal crosses	Heterogeneity between families	Deviation from expectation	Difference between reciprocal crosses	Heterogeneity between families
1	*Gd* and *Fr*.....	3	397	0	0	397						
2	*Fr* × **201,1**	1	193	0	0	193						
3	*Fr* and *Ww* ...	8	1857	141	0	1998	2,221	2,190	8,600	0,10—0,20	0,10—0,20	0,10—0,20
4	*Engl w* × *Fr*...	3	1607	120	0	1727	1,438	—	2,212	0,20—0,30	—	0,30—0,50
5	**204,1** × *Fr*	2	1109	0	74	1183	0,0001	—	0,003	0,98—0,99	—	0,90—0,95
6	*Ac* and *Fr*.....	2	263	0	20	283	0,322	0,008	—	0,50—0,70	0,95—0,98	—
7	Cdw_R × *Fr*.....	1	167	53	0	220	0,097	—	—	0,70—0,80	—	—
8	*Fr* × Cdw_N.....	1	193	56	0	249	0,837	—	—	0,30—0,50	—	—
9	*Fr* × Sl_{dH}	1	123	0	39	162	0,074	—	—	0,70—0,80	—	—
10	*Fr* × Sl_N.....	1	77	0	21	98	0,667	—	—	0,30—0,50	—	—
11	*Gd* and **201,1**...	2	758	0	0	758						
14	**204,1** × *Gd*	2	479	0	31	510	0,026	—	0,134	0,80—0,90	—	0,70—0,80
15	*Ac* × *Gd*	1	407	0	38	445	3,913	—	—	0,02—0,05	—	—
18	*Gd* × Sl_{dH}	3	169	0	49	218	0,556	—	0,993	0,30—0,50	—	0,50—0,70
19	*Gd* and Sl_N ...	3	653	0	237	890	1,260	0,185	3,040	0,20—0,30	0,50—0,70	0,05—0,10
20	*Ww* × **201,1** ...	2	536	32	0	567	0,356	—	0,518	0,50—0,70	—	0,30—0,50
23	*Ac* × **201,1**	4	221	0	14	235	0,035	—	1,803	0,80—0,90	—	0,50—0,70
24	Cdw_R × **201,1**...	1	142	49	0	191	0,044	—	—	0,80—0,90	—	—
25	Cdw_N × **201,1**...	1	237	78	0	315	0,010	—	—	0,90—0,95	—	—
27	Sl_N × **201,1**	1	161	0	54	215	0,016	—	—	0,90—0,95	—	—
29	*Ww* × **204,1** ...	1	118	0	0	118						
30	*Ac* × *Ww*	3	533	0	0	533						
32	*Ww* × Cdw_N ...	1	277	97	0	374	0,175	—	—	0,50—0,70	—	—
33	*Ww* × Sl_{dH}.....	2	143	20	10	173	6,184	—	3,458	0,02—0,05	—	0,10—0,20
34	*Ww* and Sl_N ...	5	986	251	93	1330	1,317	1,164	8,204	0,50—0,70	0,50—0,70	0,20—0,30
41	*Ac* × **204,1**	1	96	0	0	96						
42	Cdw_R and **204,1**	6	1271	296	91	1658	2,859	1,218	5,557	0,20—0,30	0,50—0,70	0,50—0,70
43	Cdw_N × **204,1**...	2	807	167	59	1033	5,499	—	0,054	0,05—0,10	—	0,95—0,98
45	Sl_N × **204,1**	1	177	0	63	240	0,200	—	—	0,50—0,70	—	—
46	*Ac* × Cdw_R.....	1	146	45	10	201	2,089	—	—	0,30—0,50	—	—
47	Cdw_N × *Ac*.....	1	95	30	12	137	2,690	—	—	0,20—0,30	—	—
48	*Ac* and Sl_{dH} ...	2	182	0	53	235	0,750	0,000	—	0,30—0,50	1,00	—
49	*Ac* × Sl_N.....	2	150	0	55	205	0,366	—	0,126	0,50—0,70	—	0,70—0,80
50	Cdw_R and Cdw_N	4	0	761	0	761						
51	Cdw_R and Sl_{dH}	3	0	235	80	315	0,027	0,370	0,490	0,80—0,90	0,50—0,70	0,30—0,50
52	Cdw_R and Sl_N	4	0	194	66	260	0,021	0,107	2,452	0,80—0,90	0,70—0,80	0,20—0,30
53	Cdw_N × Sl_{dH} ...	2	0	88	32	120	0,170	—	0,466	0,50—0,70	—	0,30—0,50
54	Cdw_N × Sl_N ...	2	0	196	56	252	0,428	—	1,029	0,50—0,70	—	0,30—0,50
55	Sl_{dH} and Sl_N...	4	0	0	157	157						

cy_2^c and cy_2^s are multiple allelomorphs of Cy_2). This distinction, which is drawn between possible genically different dwarfs, is confirmed by the fact that at least two types of dwarf may be distinguished by slight phenotypic differences. (See Fig. 1.)

Table 2 shows the crosses that were made and the reference numbers assigned to them. Table 3 gives the observed F_2 segregation and the χ^2 analysis in each case. It will be seen that, with the exception of crosses No. 15 and No. 33, the χ^2 test fails in every case to reveal any deviation from the expected totals or heterogeneity between reciprocals or within single crosses. In the two doubtful cases the deviation from expectation is not serious, and in cross No. 33 is certainly due to the low number of plants.

As appears from Table 3, cross No. 50 shows that the two crypto-dwarf types are identical. The identity between the two slender types is shown by cross No. 55. In crosses between crypto-dwarf ($cy_1cy_1cy_2^ccy_2^c$) and slender ($cy_1cy_1cy_2^scy_2^s$) F_2 should segregate into 3 crypto-dwarfs : 1 slender. In the present investigation crosses of this kind are represented by crosses Nos. 51, 52, 53 and 54, all of which gave perfect 3 : 1 segregations in crypto-dwarf : slender.

The genotype of the dwarfs was tested partly by crossing with crypto-dwarf and slender and partly by inter-crossing of different dwarfs. Dwarfs of the type of $Cy_1Cy_1cy_2^ccy_2^c$ give with crypto-dwarf an F_2 of 3 dwarfs : 1 crypto-dwarf, and with slender 12 dwarfs : 3 crypto-dwarfs : 1 slender. Crossed in a corresponding manner, dwarfs of the type $Cy_1Cy_1cy_2^scy_2^s$ give 12 dwarfs : 3 crypto-dwarfs : 1 slender and 3 dwarfs : 1 slender respectively, while $cy_1cy_1Cy_2Cy_2$ dwarfs give 3 dwarfs : 1 crypto-dwarf and 3 dwarfs : 1 slender. Finally, dwarfs of the type $Cy_1Cy_1Cy_2Cy_2$ crossed with crypto-dwarf in F_2 give 15 dwarfs : 1 crypto-dwarf, and crossed with slender 15 dwarfs : 1 slender. Crosses between dwarfs on the one hand and crypto-dwarfs and slender on the other hand are tabulated in Table 4. The observed segregation recorded in the table was re-calculated from the data in Table 3. Crosses Nos. 26 and 44, previously made by DE HAAN (1927), are marked in the table. Of the 4 possible dwarf types 3 were represented in the selected basic material. The fourth dwarf type, $Cy_1Cy_1Cy_2Cy_2$, could not be found and seems to be rare.

The analysis of the dwarfs into their genically different groups was also made by studying their behaviour on inter-crossing, and the results thus obtained fully confirm the correctness of the formulae found for

TABLE 4. *F_2 of crosses between dwarfs and crypto-dwarfs ($cy_1cy_1cy_2^{c}cy_2^{c}$) and between the same dwarfs and slender ($cy_1cy_1cy_2^{s}cy_2^{s}$).*

Parents	Cross No.	Observed segregation Dwarf : *Cdw* : *Sl*	Expected segregation Dwarf : *Cdw* : *Sl*	Formulae of the dwarf parents
$Cdw_R \times Ww$	(31)	—	3 : 1 : 0	$Cy_1Cy_1cy_2^{c}cy_2^{c}$
» × **204,1**.....	42	12,27 : 2,85 : 0,88	12 : 3 : 1	$Cy_1Cy_1cy_2^{s}cy_2^{s}$
» × *Ac*	46	11,62 : 3,58 : 0,80	» » »	» » » »
» × *Fr*	7	3,04 : 0,96 : 0	3 : 1 : 0	$cy_1cy_1C\bar{y}_2Cy_2$
» × *Gd*	(16)	—	» » »	» » » »
» × **201,1**.....	24	2,97 : 1,03 : 0	» » »	» » » »
$Cdw_N \times Ww$	32	2,96 : 1,04 : 0	3 : 1 : 0	$Cy_1Cy_1cy_2^{c}cy_2^{c}$
» × **204,1**.....	43	12,50 : 2,59 : 0,91	12 : 3 : 1	$Cy_1Cy_1cy_2^{s}cy_2^{s}$
» × *Ac*	47	11,10 : 3,50 : 1,40	» » »	» » » »
» × *Fr*	8	3,10 : 0,90 : 0	3 : 1 : 0	$cy_1cy_1Cy_2Cy_2$
» × *Gd*	(17)	—	» » »	» » » »
» × **201,1**.....	25	3,01 : 0,99 : 0	» » »	» » » »
$Sl_{dH} \times Ww$.....	33	13,24 : 1,84 : 0,92	12 : 3 : 1	$Cy_1Cy_1cy_2^{c}cy_2^{c}$
» × **204,1**	44	(DE HAAN, 1927)	3 : 0 : 1	$Cy_1Cy_1cy_2^{s}cy_2^{s}$
» × *Ac*	48	3,10 : 0 : 0,90	» » »	» » » »
» × *Fr*	9	3,04 : 0 : 0,96	» » »	$cy_1cy_1Cy_2Cy_2$
» × *Gd*	18	3,10 : 0 : 0,90	» » »	» » » »
» × **201,1**	26	(DE HAAN, 1927)	» » »	» » » »
$Sl_N \times Ww$	34	11,86 : 3,02 : 1,12	12 : 3 : 1	$Cy_1Cy_1cy_2^{c}cy_2^{c}$
» × **204,1**	45	3,15 : 0 : 0,85	3 : 0 : 1	$Cy_1Cy_1cy_2^{s}cy_2^{s}$
» × *Ac*.....	49	2,93 : 0 : 1,07	» » »	» » » »
» × *Fr*	10	3,14 : 0 : 0,86	» » »	$cy_1cy_1Cy_2Cy_2$
» × *Gd*	19	2,93 : 0 : 1,07	» » »	» » » »
» × **201,1**	27	3,00 : 0 : 1,00	» » »	» » » »

the dwarfs on crossing with crypto-dwarf and slender types. These crosses are shown in Table 5.

Cross No. 12 was made by RASMUSSON (1927) and No. 22 by DE HAAN (1927). The so-called »slender-Nilsson» type was obtained from F_2 by crossing No. 6, and the »crypto-dwarf-Nilsson» occurred in the F_2 by crossing No. 4. Both these crosses were carried out by Mr. ERNST NILSSON in 1929, who very kindly handed them over to me for further analysis.

F_2 were grown so as to make a further test of the 15 : 1 ratio of cross No. 6 and the 12 : 3 : 1 ratio of cross No. 34. The F_2 analyses, found in Table 6, fully confirm the F_2 results.

TABLE 5. *F_2 of crosses between the different lines of dwarfs.*

Parents	Cross No.	Observed segregation Dwarf : *Cdw* : *Sl*	Expected segregation Dwarf : *Cdw* : *Sl*	Formulae of the dwarf parents
Engl.w × *Fr*	4	14,89 : 1,11 : 0	15 : 1 : 0	$Cy_1Cy_1cy_2^ccy_2^c \times$
Ww × *Fr*	3	14,87 : 1,13 : 0	» » »	$cy_1cy_1Cy_2Cy_2$
» × *Gd*	12	(RASMUSSON, 1927)	» » »	
» × **201,1**	20	15,10 : 0,90 : 0	» » »	
204,1 × *Fr*	5	15,00 : 0 : 1,00	15 : 0 : 1	$Cy_1Cy_1cy_2^scy_2^s \times$
» × *Gd*	14	15,03 : 0 : 0,97	» » »	$cy_1cy_1Cy_2Cy_2$
» × **201,1**	22	(DE HAAN, 1927)	» » »	
Ac × *Fr*	6	14,87 : 0 : 1,13	» » »	
» × *Gd*	15	14,63 : 0 : 1,37	» » »	
» × **201,1**	23	15,05 : 0 : 0,95	» » »	
Ww × **204,1**	29	1,00 : 0 : 0	1 : 0 : 0	$Cy_1Cy_1cy_2^ccy_2^c \times$ $Cy_1Cy_1cy_2^scy_2^s$
Ac × **204,1**	41	1,00 : 0 : 0	1 : 0 : 0	$Cy_1Cy_1cy_2^scy_2^s \times$ $Cy_1Cy_1cy_2^scy_2^s$
201,1 × *Fr*	2	1,00 : 0 : 0	1 : 0 : 0	$cy_1cy_1Cy_2Cy_2 \times$
» × *Gd*	11	» » »	» » »	$cy_1cy_1Cy_2Cy_2$
Fr × *Gd*	1	» » »	» » »	

TABLE 6. *Table showing F_3 analysis of Crosses Nos. 6 and 34.*

Families raised from	Constitution of the F_3 families Dwarf : *Cdw* : *Sl*	Number of families observed	Number of families expected	χ^2
Cross No. 6.....	1 : 0 : 0	40	40,6	0,009
	3 : 0 : 1	25	23,2	0,140
Dwarf F_2 plants	15 : 0 : 1	22	23,2	0,062
	Total	87	87	0,211
Cross No. 34 a	1 : 0 : 0	19	22	0,409
Dwarf F_2 plants	12 : 3 : 1	21	22	0,045
	3 : 1 : 0	15	11	1,455
	3 : 0 : 1	11	11	0,000
	Total	66	66	1,909
Cross No. 34 b	0 : 1 : 0	15	13	0,308
Crypto-dwarf F_2 plants...	0 : 3 : 1	24	26	0,154
	Total	39	39	0,462

	χ^2. Deviation from expectation	Probability for deviation from expectation
Cross No. 6	0,211	0,90
Cross No. 34 a	1,909	0,50—0,70
Cross No. 34 b	0,462	0,30—0,50

All the crosses made show the complete agreement between the results obtained and those expected on the multiple allelomorph theory. The final result of the investigation of the length factors in the *Pisum* lines examined is represented in Table 7, which gives the genotype of the 11 different lines, assuming the theory advanced in the first section, along with the formulae used by RASMUSSON and DE HAAN.

TABLE 7. *Genotypic notation of Pisum lines investigated in this work according to method of annotation used in this and previous investigations.*

No.	Symbol	Genotype	RASMUSSON	DE HAAN
1	*Fr*	$cy_1cy_1Cy_2Cy_2$		
2	*Gd*	» » » »	$cry_1cry_1Cry_2Cry_2$	
3	*201 . 1*	» » » »		*la la Lb Lb*
4	*Ww*	$Cy_1Cy_1cy_2{}^{c}cy_2{}^{c}$	$Cry_1Cry_1cry_2cry_2$	
5	*Engl. w*	» » » »		
6	*204 . 1*	$Cy_1Cy_1cy_2{}^{s}cy_2{}^{s}$		*La La lb lb*
7	*Ac*	» » » »		
8	Cdw_R	$cy_1cy_1cy_2{}^{c}cy_2{}^{c}$	$cry_1cry_1cry_2cry_2$	
9	Cdw_N	» » » »		
10	Sl_{dH}	$cy_1cy_1cy_2{}^{s}cy_2{}^{s}$		*la la lb lb*
11	Sl_N	» » » »		

Linkage. — No linkage was detected between either of the *cy* loci and the other factors mentioned in the second section.

One more linkage was tested, viz. that of the cy_2 locus and the factor which gives emerald plants. DE HAAN (1930) found complete linkage between these two loci. Cross No. 4 of the present investigation was between English wonder mutant, which is emerald, and Frühe niedrige. The F_2 was difficult to score for emerald, but there was a strong indication of linkage between the cy_2 locus and that of *w*. According to ERNST NILSSON the factor for emerald in the English wonder mutant was *wlo* (ERNST NILSSON, 1933).

Discussion. — The occurrence of polymeric factors in a truly diploid type as *Pisum* might be due to duplication, as suggested by DE HAAN (1932). I am now planning a more thorough investigation of the linkage relation between the *cy* loci and certain other loci of a polymeric or possibly polymeric nature.

DE HAAN (1934) proved that the *cy* factors were truly polymeric, and thus refuted the hypothesis of SIRKS (1926) and OELKERS (1930),

explaining such cases by assuming multiple allelomorphism. The present investigation shows that with respect to the length factors in dwarf peas polymeric as well as multiple allelomorphic factors are concerned.

I wish to express my indebtedness to Professor H. NILSSON-EHLE, whose ready courtesy rendered this investigation possible, to Dr. J. RASMUSSON for material and for helpful advices, to Mr. ERNST NILSSON for material, to Messrs. K. G. v. SYDOW and Å. NYHLÉN for great assistance in the field work, and to Dr. K. MATHER for advice as to the statistical treatment of the data.

SUMMARY.

1. The present work indicates that two loci have mutant allelomorphs affecting the expression of dwarf peas. The factor cy_1 appears to be common to both the crypto-dwarf and the slender modification of dwarf. The other locus appears to carry two mutant genes cy_2^c and cy_2^s which together with cy_1 gives crypto-dwarf and slender plants respectively.

2. 11 lines, 7 of which are commercial varieties, comprising 7 dwarf, 2 crypto-dwarf and 2 slender types, gave F_2 segregation on intercrossing, thus fully confirming this theory. The factorial constitution of these lines is given.

3. No linkage was detected between either of the *cy* loci and those of *r*, *tl*, *i* and *a*.

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INHERITED SEX-MOSAIC IN MAN

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I. INTRODUCTION.

IN a paper by LUNDÉN (1918), a case is described where a woman, who was operated upon for inguinal hernia, was found to be an intersex. A sister of this woman, also suffering from inguinal hernia, was found at the operation to be an intersex of the same type (STRANDMAN, 1918). Finally, one of the present authors (PETTERSSON, 1936) performed an operation on a daughter of a third sister, this girl also being an intersex of a similar type. It is evident that this intersexuality is of an hereditary nature, and it shows points of interest within the fields of genetics and medicine. In the following report sections II, III and IV are written by PETTERSSON, the others by BONNIER.

II. DESCRIPTION OF THE NEW CASE.

This case, No. 1231 of the genealogical table, Fig. 1, may be briefly described as follows:

Journal No. 1241/35, 14 years old. Hitherto always healthy. For the past 1 1/2 months a hernia was observed in the right groin. No pains in the hernia. No incarceration. *Condition upon admission*: In the right groin there is a small hernia, in which a body the size of a walnut may be felt (ectopic ovary?). The body cannot be replaced into the abdominal cavity. *Operation* (PETTERSSON): Evipan anaesthesia. After an ordinary inguinal incision the hernial sack was opened. This was of the same appearance as a tunica vaginalis in congenital hernia. The hernial sack was in open communication with the abdominal cavity. The contents of the hernial sack looked like a testis of normal size for the age, together with the epididymis and the vas deferens. As it seemed probable that some kind of intersexuality was present, laparotomy was made in the median line in order to examine the interior sexual organs. It was then found that the uterus, as well as the adnexa, were entirely absent. At the left pelvis border there was a testis-like body, somewhat smaller than the one on the right side. Both the sexual glands were connected by means of cord-like growths with a low transversal fold below the bladder. By feeling with a finger it was ascertained that only a narrow septum separated the blindly ending vagina from the abdominal cavity. The abdominal wound was closed. The partly descended testis on the right side was extirpated, as there seemed to be a risk of torsion or of malignant degeneration if it was replaced into the abdominal cavity. The inguinal wound was closed.

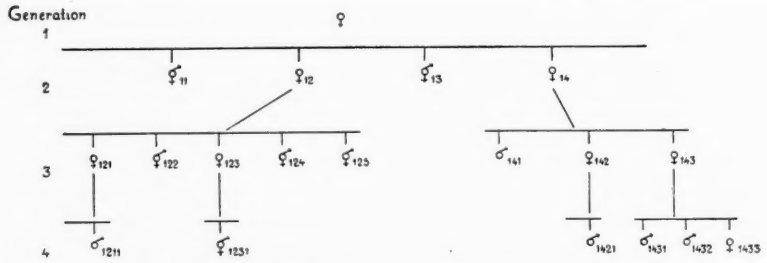

Fig. 1. Genealogical table of a Swedish intersexual family.

Fig. 2. Section through testis of Case 1231 of Fig. 1.

Pathological-anatomical examination (SNELLMAN): Total weight of the specimen 40 gr. The ovoid body is 21 × 13 × 14 mm. On one side a hilus-like area is observed. Along one of the borders there is a string about 3 mm. thick. This ends in a club-

shaped swelling at one end of the ovoid. A swelling is also discernable at the other end. — On microscopic examination (Fig. 2) the ovoid body is seen to be a testis. The ducts are mostly separated by connective tissue, which is rather abundant, with solitary collagenous fibres or small groups of such and with groups of interstitial cells. In some places, however, the walls of the ducts are close to one another. The epithelium of the ducts is built up of Sertoli's cells. Some of the epithelium is single-layered with basal nuclei. A considerable part is apparently multi-layered, however, and some of it has evidently several layers. Spermatogenesis is perhaps beginning. In a few of the ducts small lumina are seen. The club-shaped swelling at one of the poles is the epididymis together with a duct with a heavily folded mucous membrane, rather high cylindrical epithelium and tuba-like structure.

After the findings of the operation the journal was completed with the following data: Exterior typically female. The knees are not in valgus position. The face of normal size. The Adam's apple is not protruding. The breasts are normal for the age with projecting conical nipples. The pubic hairs are not developed. The labia majora are rather small. The labia minora reach outside of the lab. maj. The clitoris is somewhat larger than normal, imperforate and without an inferior furrow. The orifice of the urethra is $^1/_2$ cm. in front of the introitus vaginae. Hymen pliable. Inside the hymen there is a vagina, about 3 inches wide and $1\ ^1/_2$ inches deep, with a blind ending.

The description of the case was also completed with the following data. The girl is the only child. Her interests have always been typically female and she has the manners of a girl. When she was 3—4 years old, her father observed that the clitoris was larger than usual. He intended consulting a physician but forgot the whole thing.

III. DESCRIPTION OF THE TWO PREVIOUS CASES IN THE SAME FAMILY.

♀ 125. Examined by LUNDÉN (1918) who reports: N. N. 21 years old. Has never menstruated but has had regular nasal bleedings. Hernia since 10 years old. 3 days before the admittance to the hospital she had felt pains and a tender tumour in the left groin. *Condition upon admission*: In the left groin there is an immovable hard swelling, the size of a hen's egg. Symmetrically, in the right groin there is a fairly hard body, the size of a walnut, which is easily moved down towards the anterior part of labia majora and also a short distance into the inguinal canal. The case was diagnosed as inguinal hernia on both sides, containing the ovaries, on the left side combined with torsion, incarceration or inflammation. A gynecological examination showed that the vagina ended blindly. On palpation through the rectum neither ovaries nor uterus could be felt. On either side there was a string towards the bladder. The right hernial sack, which was opened at the operation, was found to be completely closed and resembled a tunica vaginalis. It ended blindly in the inguinal canal and reached down into the upper anterior part of labium majus. The contents were exactly like a testis, somewhat undersized for the age, together with the epididymis and the vas deferens. The latter was followed up to the region of the inner inguinal ring. The peritoneum was opened. On palpation the total absence of uterus and adnexa was verified. The further course of the vas deferens-like formation could not be ascertained, but it was considered probable that the

strings, felt on rectum-palpation to run towards the bladder, were identical to it. The testis-like growth was replaced into the abdominal cavity and the wound was closed. On the left side the operation showed a similar tunica vaginalis and the contents were also similar, but the epididymis was reddened, swollen and hard. On this side an ablatio testis was performed and then the wound was closed.

Pathological-anatomical examination (FORSELIUS) of the specimen showed, among other things:

The growth weighs 35 gr. and consists of a fusiform body, $6 \times 2\,^1/_2 \times 2$ cm., embedded in a white shining capsule, harder and more yellowish white in the upper 2 cm. The cut surface in the lower 4 cm. shows the typical section of a testis, greyish white, of a small-granulated texture, parenchyma soft, elastic. The upper 2 cm. contained an abscess the size of a bean. Under the microscope a typical testis structure was evident. The individual tubules of the seminiferous type have typical Sertoli's cells within the membrana propria, besides spermatogonia and spermatocytes, but no typical spermatids and no spermatozoa. The tubuli recti were of usual structure. The interstitial connective tissue was well developed. The interstitial cells were unusually prominent. The abscess above the testis belongs to the epididymis. The structure is without any specific features. The rest of the epididymis shows small canals of the usual type, without any spermatozoa. The vas deferens has a thick muscular wall and around a star-shaped lumen a mucous membrane, covered by a transitional epithelium.

After the operation the journal was completed with the following data: Typically female exterior, without any masculine traits, although the shoulders are unusually broad and the knees not placed in valgus position. The face is small and girlish, the hair long and thick. The voice is typically female and the Adam's apple not protruding. The breasts are large with a normal areola, the nipples are not prominent. The upper border of the pubic hairs is transversal. Labia majora normal, labia minora somewhat small, covered by labia majora and not visible until the latter are drawn sidewise. Clitoris rather small, imperforate and without an inferior furrow. The orifice of the urethra in the normal place $^1/_2$ cm. in front of the introitus vaginae. Hymen low, pliable. Inside of hymen a blind pocket the size of a finger-end. On rectum-palpation the replaced right testis is felt near the pelvis wall, in addition to the vasa deferentia already mentioned.

The patient was advised not to marry. For some time she worked in an office and then wanted to become a medical gymnast, but had to discontinue her education owing to nasal bleedings. The patient has now been *married* for several years in the U. S. A.

♀ 124 was examined by STRANDMAN (1918) who reports: N. N. 27 years old. Has had a left sided inguinal hernia since childhood, and for some time another on the right side. The left hernia extended down to the upper part of labium majus, the right one protruded as large as a walnut at the inner inguinal ring. In both hernias a soft tumour about the size of an almond was felt, which could easily be moved into the inguinal canal but returned immediately. The patient had never menstruated and had not had any nasal bleedings. Her type was pronouncedly female and the facial expression, the voice, movements and stature were those of a woman. The breasts were well developed but the nipples were not projecting. The pubic hairs were sparse. The exterior sexual organs normal except for a very narrow opening in the hymen. The vagina ends blindly 3 cm. behind the hymen.

On rectum-palpation a fold across the pelvis was felt, but no uterus. During the operation testes were found in both hernias. Both the testes were rudimentary, somewhat smaller than an ordinary ovary. They were replaced into the abdominal cavity and no pathological-anatomical examination could be made.

The patient was advised against marriage. She managed her business with energy and was very interested in sport, which was considered to point to a certain degree of manliness in her character. Her mother asserted that she had never been like other girls, had never been interested in male company and never thought of marriage, although suitors had not been lacking (acc. to STRANDMAN). Obviously the patient has changed her mind, for she *got married* several years ago.

IV. RESULTS OF AN INVESTIGATION OF THE FAMILY.

♂? 11. Unmarried. Uncertain information as regards menstruation. According to certain statements she has never menstruated. She is now more than 80 years old and very weak, hence an examination is impossible.

♀ 12. Normal. Married. Unrelated to her husband.

♂ 13. Unmarried. Hernia since her childhood. Has not menstruated, but has had nearly regular monthly nasal bleedings until 50 years old. Female exterior and the interests of a woman. Has been a school-teacher. Refuses examination and explains that she believes in the »restoring and equalizing powers of Nature».

♀ 14. Married. Had not menstruated until after the birth of her first child. Previously she had regular nasal bleedings. Has normal children and grandchildren.

♀ 121. Normal. Married. Has a married son, but so far no grandchildren.

♂ 122. Unmarried. Hernia from childhood. Has not menstruated. Female exterior and the interests of a woman. Has not been interested in male company and not considered marriage, although suitors are said not to have been lacking. Works in an office. Refuses to be examined, maintaining that the question of her sex is of a private nature.

♀ 123. Normal. Married. Unrelated to her husband. Mother of 1231.

V. INTERSEXES OF A SIMILAR TYPE, RECORDED IN LITERATURE.

It has probably been known since the oldest times that hermaphrodites — i. e. individuals with male as well as female characteristics — occur. In antique art hermaphrodites are common, in sculpture and in pictures: usually the are pictured with the head and upper torso of a female but with the external genitals of a male. It is known, however, that such monsters are very rare, and probably the old pieces of art were inspired more by the imagination of the artist than by his actual knowledge. More or less verified cases of hermaphroditism are known from the Middle Ages and from the 16th—17th centuries, but fully verified cases and scientific descriptions are restricted to a more recent period. It is practically impossible to determine the number of verified

cases of hermaphroditism in Man, since the descriptions are spread over a very large number of publications and often consist only of brief notes. Most probably, however, the number exceeds 2000.

Modern comprehensive treatises in the field deal mainly with the theoretical problems (see especially BERNER, 1930), whereas the latest descriptive monographs of known cases date from the beginning of this century. In the first line the large monograph by v. NEUGEBAUER (1908) should be mentioned. The author includes hypospadias in hermaphroditism, and, therefore, his monograph includes several cases, which really do not belong there, but this error does not seriously diminish the value of his book. Among treatises of a somewhat later date one may be mentioned, namely that by POZZI (1911), which mainly includes reports of his own cases, but also observations made by others. It would, however, no doubt be of great value in medical as well as in general biological sex-research if a work, bringing that of v. NEUGEBAUER up to date, was available. Owing to the lack of such a work it is possible that the review of certain cases, given below, is not so complete as it should be.

In going through the literature one finds that the cases of hermaphroditism or intersexuality described vary from nearly female individuals to nearly male individuals, with a large number of transitional types. It is then quite natural that in earlier days the attempts to systemasize the intersexes were made from an anatomical point of view, and that several different systems were suggested. It was not until GOLDSCHMIDT's studies of the heredity and embryonic development of intersexes among insects that an attempt was made to classify the intersexes in general — even the human — from the point of view of developmental physiology (cf. GOLDSCHMIDT, 1931). According to GOLDSCHMIDT, human hermaphrodites are to be considered as female intersexes (cf. the discussion of this question in section VII of the present paper), disregarding their anatomical characters: the different anatomical categories correspond to different degrees of female intersexuality.

In looking over the wide variety of human intersexes we find, however, one group that seems to be fairly well defined, i. e. the different cases within this group are very similar in essential anatomical characters. It is possible that this group just corresponds to a certain *degree* of intersexuality, but, on the other hand, it seems justified to assume, that the developmental physiology has been similar in all cases within this group. In other words: this anatomically well defined group

is probably also rather well defined from a physiological point of view. The group in question is that, to which the cases described in sections II—IV of this paper belong, and the main characteristics are the following:

External anatomical: General appearance typically female; Adam's apple, voice, hairiness, breasts female. No trace of a beard. Vagina brief and ending blindly. Clitoris generally normal, sometimes a little oversized.

Internal anatomical: Internal sexual organs of the female completely absent, neither ovaries nor remainders of the Müllerian ducts are found. Testes with epididymis and vas deferens are present. The descent may be completely lacking or has advanced more or less. In most cases the testes are situated in hernial sacks. In all cases, when the testes have been microscopically examined they consist of typical testicular tissues, the interstitial tissue often being hypertrophied, and with a more or less advanced spermatogenesis, in some cases even with spermatozoa.

Physiological: Menstruation not occurring. (In some cases, menstruation is said to have occurred. If these reports are correct, however, the absence of a uterus makes it evident that it cannot have been a real menstruation, but some kind of a vicarious one.) In some cases the menstrual molinomina occur, sometimes connected with a vicarious menstruation, such as nasal bleedings.

Mental: Interests and general temperament are female. The individuals always consider themselves as women, and no doubt regarding the sex has arisen until medical treatment for inguinal hernia has become necessary (apart from suspicions aroused by the non-existent menstruation).

Sexual: Their sexual instinct is never that of a male, sometimes it is perhaps indifferent but often typically that of a woman.

It is unnecessary to describe all the cases we have found in the literature belonging to this group. Among those cases, however, there are some which belong to one and the same family, and where it is probable that the phenomenon is inherited. Those cases seem to be of great interest in connection with the similar data presented in the present paper, and a brief description will be given in chronological order and including only such details as are necessary to complete the general description given above.

LEOPOLD (1875) described a farmer's wife of 50 years age, happily married for 25 years. Womanly. Sparsely haired on mons veneris. She was treated for hepatical carcinoma. She had two married sisters. who like herself had never menstruated and had no children.

DIXON JONES (1890) operated upon a girl of 21 for double inguinal hernia. No menstruation or vicarious menstruation. Typical woman, although the larynx was more protruding than normally. Clitoris somewhat small. As regards the family of the patient DIXON JONES writes: »The patient's mother had eight daughters, two of whom were affected in a similar manner. Dr. S. G. WEBBER, of Jamaica Plains, Mass., wrote me, that one of them had never had her catamenia and had

congenital absence of the uterus». It is not quite evident, if DIXON JONES's patient was one of the two sisters »affected in a similar manner», but this seems to be the most probable interpretation of his statement. Anyway, the difference has no fundamental importance for our final discussion. No brothers are mentioned, but as not only the abnormal but also her normal daughters were mentioned, we conclude that there were 6 normal women and 2 intersexes in the family.

MARTIN (1894). A nurse of 20, who had never menstruated, was operated upon for a hernia on the right side. A body was found, supposed to be an ovary, and was replaced into the abdominal cavity. Later on she was operated upon for a swelling on the left side. External genitals: no hairs, clitoris normal. The swelling was extirpated and found to be a testis, which included spermatogenesis in different stages. In a few tubuli, states MARTIN, »imperfect spermatozoa may be distinguished». A sister of the patient, two years older, had the same characteristics: no menstruation, infantile breasts, no pubic hairs, vagina ending blindly, no sign of the uterus. This sister was not operated upon.

HENGGE (1903) describes two sisters, treated by MARTIN. The younger was 19 years old and had never menstruated. Since the age of 14 she had had headaches and nausea one day every fourth week, and until 4 weeks before the operation these molinomina were associated with repeated nasal bleedings. Then the bleedings ended, but the molinomina were repeated every week, and her capacity for work was seriously impeded. The mons veneris and the armpits without hairs. Clitoris normal. The testes in the labia majora were extirpated and microscopically examined. As regards the seminiferous tubules HENGGE states: »Although the epithelium, present in several layers, is quite well developed, spermatozoa are nowhere developed». The elder sister (32 years) had been married for 9 years. External appearance and genitals as in the younger sister (blind-ending vagina, unhaired mons veneris a. s. o.). On palpation a body is felt in the right labium, which is similar to a testis. There is also a small growth in front of the left inguinal ring which may be moved into the hernial canal. No internal female sexual organs are felt. Regular coition, only occasionally with libido. The two sisters were the oldest and the youngest of 6 children, there being another 3 sisters and one brother. Of the 3 sisters two were married and had 3 and 2 children respectively, the unmarried sister had regular menstruation. The brother was married and had two children.

DIEFFENBACH (1912) examined a girl of 19 who had double inguinal hernia of the same general type as that already described. Clitoris about 2 cm. Never menstruated. As a child she had had more of a boy's interests than those of a girl. Love had not played an important rôle in her life but she had promised a man to marry him. She now wanted to be operated upon, in order to become normally suited for marriage. The operation showed testes in the inguinal canal and the microscopical examination showed typical testicular tissue with spermatogenesis and even spermatozoa.

DIEFFENBACH studied the family and found a large number of females who had never menstruated. The result of his study is summarized in the genealogical table in Fig. 3. The individual marked A is the patient just described, those denoted with ♀ are women, who have not menstruated and are supposed to be intersexes. Two of those in generation 3 had been married without children, two in generation 4 had had »genital abnormalities». The individual marked B was a girl of 7 who was examined but not operated upon. Her clitoris was 4 cm. and she lacked a vagina.

The table includes some individuals of uncertain sex. If we count the »probably ♂» as a male and exclude the 11 individuals who died in infancy and the 6 aborted ones we find that among the children of those women who have carried the abnormality to the next generation there are 10 women, 8 intersexes and 5 men.

LOENNECKEN (1922) operated upon two sisters for double inguinal hernia. One of them was 21 years old and had never menstruated, the other one was 13. In the hernial sack there were testes and an epididymis. No trace of ovaries or uterus. External genitalia female but vagina only half a finger's length and ending blindly. Breasts well developed. »Looking more closely» at the sisters, since the examination was made, they gave a »somewhat more mannish impression». The testes were not

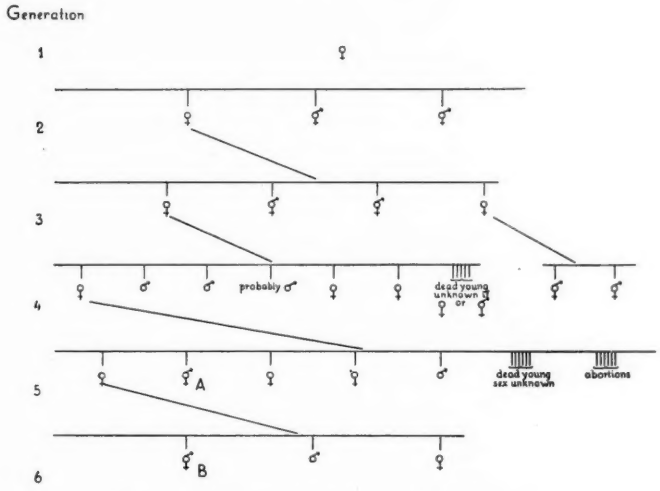

Fig. 3. DIEFFENBACH's intersexual family table.

extirpated and no microscopical examination could be made. Later on a third sister was operated upon for double inguinal hernia. She was 25 and had never menstruated and showed the same symptoms as the other sisters. She had marked features and strong hands and legs, the thighs were more of a male than of a female. The testis was extirpated and examined. The tissue was that of a normal testis, showing spermatogenesis up to spermatogonia and spermatocytes. These sisters were not married or engaged, and seemed to be sexually indifferent. There was a fourth sister with double inguinal hernia, who expected to be operated later on. In all there were 9 sisters and two boys. If the sisters not mentioned above are counted as normal we obtain 5 women, 4 intersexes and 2 males.

Finally, in the new case described here, we find (provided we consider, as above, women without menstruation to be intersexes) among the progeny of females to whom the abnormity has been transmitted, 4 women, 6 intersexes and no men.

VI. GENETICAL DISCUSSION.

In discussing the inheritance of the sexual abnormality described here we consider it justifiable — for reasons given in the previous section — to pool the data from our own case with those of hereditary nature, reported by previous authors. All the cases reviewed in the last section do not, however, give sufficient information about all members of the families. A fair certainty concerning the whole families exists in the cases recorded by DIXON JONES, HENGGE, DIEFFENBACH and LOENNECKEN and in our own case. In the genetical discussion we shall only use these latter cases, pooling their data. It may be remarked that even if these cases are fundamentally alike anatomically and physiologically, it does not necessarily follow that the same gene (or genes) is responsible in the different families. Many cases are known, where different genes cause the same or very similar characters. It seems probable, however, that even if the same gene is not responsible in all the cases, the different genes are at least allelomorphic. At any rate, in comparing the likelihood of different hypotheses it is permissible to presume one and the same gene as responsible in all the cases. Our discussion will deal largely with the abnormal sex-ratios, and it is evident that the likelihood of a certain hypothesis is the same, whether these ratios have been caused by one and the same gene in all cases or by a series of different genes with similar effects.

Two groups of frequencies are essential to the analysis, which we attempt to make:

1. The frequency of male and female transmitters, i. e. individuals transferring the gene or genes in question without being visibly affected. Such frequencies may be obtained only in cases where the family-analysis is carried over several generations, i. e. only in DIEFFENBACH's and in our own material. The result is summarized in Table 1.

TABLE 1.

Case	Number	
	♀	♂
DIEFFENBACH	5	—
Our own	2	—
Total	7	—

2. The frequency of women, intersexes and men within such progenies where intersexes occur, or which are the direct issue of

verified transmitters. The result is given in Table 2, with due reservation, because of the somewhat insufficient data in some of the cases taken from the literature.

TABLE 2.

Case	Number		
	♀	⚥	♂
DIXON JONES	6	2	—
HENGGE	3	2	1
DIEFFENBACH	10	8	5
LOENNECKEN	5	4	2
Our own	4	6	0
Total	28	22	8

From Table 1 it is evident that only women and not men may be transmitters of the gene or genes for the abnormality in question. The material is small, but it proves definitely that male and female individuals are not equal as transmitters. If this were the case the probability of the frequencies in Table 1 would be $(^1/_2)^7 = 0{,}0078$ or such a small probability that the possibility may be excluded. If there is a difference between the sexes as regards the ability to transmit the gene to future generations, it is most consistent with generally accepted genetical theorems to suppose that only the females may act as transmitters, any other assumption would imply the use of more or less far-fetched auxiliary hypotheses. In the following we shall therefore assume that only females may act as transmitters of the gene or genes causing the abnormality.

In Table 2 it will be seen that there are altogether too few males. This fact, as well as the frequencies in Table 1, might be easily explained by the assumption that the sex-mosaics are triploid intersexes of the *Drosophila*-type (cf. BRIDGES, 1934). In such a case the female carriers should be triploid in all chromosomes, whereas the intersexes should be triploid in all or most of the autosomes and have two *X*-chromosomes as normal diploid females. In the great number of such cases in *Drosophila* there is always a considerable deficiency of males. For instance, in a certain series of experiments (BONNIER, 1933) the progeny of 1035 triploid *Drosophila*-females consisted of 39555 females (9273 of which were triploid), 15351 intersexes and 8078 males. These proportions are not exactly the same as in Table 2 (the χ^2 test indicates

that there is a probability of 0,05 that the proportions in Table 2 are random samples of the proportions in the *Drosophila*-case), but it is not necessary to assume that *Man* and *Drosophila* must show exactly the same results. According to the *Drosophila*-experience, however, the intersexes, should not be similar, but vary within rather wide limits. Still more important is the fact that the *Drosophila*-intersexes suffer from serious abnormalities in several different organs and generally have a low viability, an effect which is to be expected from the disturbed genic balance. Such an effect is also to be expected in all kinds of aneuploids. The human intersexes dealt with in this paper are, however, normal in all other characteristics than that of sex, and apparently they have a normal viability. The triploidy-hypothesis is the only one capable of explaining the frequencies in Table 2 without the aid of auxiliary hypotheses, but until further facts in its favour are brought forward we consider it, for reasons just mentioned, necessary to reject this hypothesis.

Thus, if we must conclude that all members of the families in question are diploid, the intersexual individuals must be genetical men. Among 58 individuals 29 ought to be females and 29 men. The chance that not more than 8 genetic males occur in a random sample of 58 taken from a population with the proportion 1 : 1, is equal to the sum of the last 9 terms in the expansion of $(^1/_2 + ^1/_2)^{58}$ or 0,00000000785 which is so small a value that the possibility may be completely disregarded. If, on the other hand, the intersexes are assumed to be genetically males, the proportion is 28 females to 30 males, which fits the expected 29 : 29 excellently.

The genical explanation of the data observed offers the possibility of several alternative hypotheses. It is possible that the intersexes are the result of several cooperating genes (non-existent in the normal population). The female carriers in DIEFFENBACH's and our own material seem not, however, to have been related to their husbands. It is, therefore, most probable that the necessary genes were all carried by the transmitters and in such a case the data point to one gene only as responsible in each of the different cases. Below we shall, therefore, assume only one gene. (Several, strongly linked genes will, in a small material, give the same apparent effect as one single gene.)

Alternative 1: *Autosomal gene.* — According to this hypothesis the gene cannot be completely dominant, since the carriers are normal women. On the other hand it cannot be completely recessive, since the occurrence of homozygotes is practically impossible in a mating

between unrelated parents. This hypothesis, thus, implies the assumption of a variably prevalent gene *A*, with *Aa* sometimes normal, sometimes intersex. Further, we must assume that the effect of the gene is sex-limited, since all the carriers are female. Together with *XX*, *Aa* gives in general normal individuals, together with *XY* it causes intersexuality in most cases. This hypothesis leads us, quite independently of the data in Table 2, to the assumption that at least most of the intersexes are genetical males. And since it seems very improbable that some of them should be males, others females, we are again forced to the conclusion that the intersexes are *XY*-individuals.

Alternative 2: *Sex-linked gene.* — According to this hypothesis the gene cannot be completely dominant, but the assumption of its being recessive fits the actual data excellently. If the gene is *a* the normal carriers should be X_AX_a and the intersexes X_aY. Thus, even this hypothesis implies the assumption that the intersexes are genetical men.

Alternative 3: *Plasmatic gene.* — This alternative is taken directly from GOLDSCHMIDT's *Lymantria*-investigations (cf. e. g. GOLDSCHMIDT, 1935). Since the intersexes are genetical men and their fathers belong to the normal population, their mothers being carriers, it should be assumed, that these transmitters have a female-factor in the plasm, $\boxed{F}$, which is considerably stronger than the one in the normal population. But then *all* the genetical sons of a carrier should be intersexes, and *all* her daughters carriers. Since none of these consequences is fulfilled in the actual data, this hypothesis must be rejected. GOLDSCHMIDT (1931) himself is of the opinion that in Man, where the female is homogametic, the femininity genes, *F*, are situated in the *X*-chromosome, and the masculinity genes, *M*, in the autosomes. There is then no special reason for assuming plasmatic genes in this case.

As an explanation of family tables of DIEFFENBACH's type GOLDSCHMIDT (1931) suggests that the husband of the original female in the table is a genetical woman, thus having *XX*. It must then be assumed that the male-determining genes of the autosomes are very potent, since the change in sex is complete. At the same time both the *X*-chromosomes must contain female-determining genes of different potency. In the progeny of such a male *XX*-individual all individuals will be *XX*. From their father some of them have inherited a potent *M*-factor and may, thus, develop into men, and the different potency of the *F*-genes in the *X*-es makes it possible that also intersexes and females develop. According to this hypothesis, the intersexes are genetical women, an assumption necessary for GOLDSCHMIDT, who

denies the possible existence of intersexuality of a hereditary nature occurring among genetical men. (Cf. the discussion in section VII below.) This hypothesis, however, does not account for the frequencies of Table 2 and must be rejected.

It is also possible, that the original female in the family table has *X*-chromosomes with unusually weak *M*-genes. This assumption is, however, equivalent to alternative 2 above from the standpoint of the mechanism of the inheritance of the abnormality.

Of the hypotheses advanced here only 1 and 2 seem to fit the actual data. It is impossible to decide which of these is the correct one. Since, however, alternative 1 requires the auxiliary hypothesis that the effect of *Aa* is sex-limited, whereas alternative 2 does not require a corresponding auxiliary hypothesis, we consider the assumption of a sex-linked gene to be the most probable one.

From another point of view, however, all the above hypotheses (except the one assuming triploid females) require an additional assumption, if they are to fit the data in Table 2. Whether the gene in question is autosomal or sex-linked the result should be an equal number of normal males and intersexes. The probability of not more than 8 occurring as a random sample of 30 taken from a population with the proportions 1 : 1 (15 : 15) is equal to the sum of the last 9 terms in the expansion of $(^1/_2 + ^1/_2)^{30}$ or only 0,0008049 or so small that we are forced to adopt an auxiliary hypothesis. (If it had not been necessary, for several reasons, to reject the triploidy hypothesis, there would have been no need of an extra hypothesis. The probability that 22 : 8 is a random sample of the proportion intersex : male in the *Drosophila*-example mentioned, viz. 15351 : 8078, is more than 0,3 or very great.) Two auxiliary hypotheses are possible. According to one of them equal numbers of zygotes with and without the abnormality gene are formed, but some of the genetically normal zygotes nevertheless develop into intersexes; the prematuration hypothesis. According to the other hypothesis the chromosomes are not distributed at random.

Prematuration hypothesis. — Presuming that the intersexuality gene is sex-linked, called *a*, this hypothesis implies the assumption that some of the genetically normal X_AY men occur among the intersexes. The cause should be that *a* exerts an influence upon the cytoplasm during the ovogenesis and previous to the ripening of the eggs in the X_AX_a individuals, resulting in the possibility that X_A as well as X_a eggs develop into intersexes. If the egg is fertilized by an X_A spermatozoon from a normal male, the abnormal tendence is suppressed, and all

females develop normally. If the fertilization is brought about by a *Y* spermatozoon, however, there is nothing to counteract the tendency, and X_aY individuals always, X_AY individuals sometimes, become intersexual. A hypothesis of this kind has been put forward several times in the genetical literature. The first instance seems to be, when MORGAN (1912) used it to explain certain cases of sterility in *Drosophila*, and later CLARA LYNCH (1919) used it for the same purpose. At first GOLDSCHMIDT explained the results of his *Lymantria*-experiments alternatively by the hypothesis that the female-determining factor was situated in the *Y*-chromosome and influenced the cytoplasm previous to the ripening of the egg. Later on (1933) he proved that this factor is located in the cytoplasm itself, but the other hypothesis is not in itself absurd. By the aid of this hypothesis it might also be possible to explain why the woman 14 had not menstruated before her marriage, but had vicarious bleedings, without assuming her to be a carrier, that is to say heterozygous X_AX_a (an assumption that does not fit the data too well). We should then assume that the gene *a* of her mother had so influenced the cytoplasm of the eggs before their ripening, that in spite of the fact that an X_A egg developed and was fertilized by an X_A spermatozoon, the resulting female X_AX_a (14) showed evidences of the influence of *a* and was amenorrhoeic. This explanation, however, does not seem very probable, since it implies that the evidences of the cytoplasmatic effect of *a* in this woman should be more potent than the actual presence of *a* in the heterozygotic carriers. As a matter of fact, it seems very difficult to find a plausible explanation of the amenorrhoeic nature of the woman 14 prior to her marriage.

If alternative 2 should not be true and an autosomal gene *A* instead being the cause of the intersexuality, this does not influence the applicability of the prematuration hypothesis. It is only necessary to assume that before the ripening of the eggs of an *Aa* individual, *A* so influences their cytoplasm that some normal *a* eggs, after fertilization by an *aY* egg, develop into intersexes.

Selective chromosome distribution. — According to this hypothesis the different chromosome combinations are not formed at random at meiosis. If we assume a sex-linked gene *a*, the assumption should be that the X_a chromosome gets into the egg more often than the X_A, or, which amounts to the same thing, that of the two, X_A most often gets into a polar body. Evidently the result should be more intersexes than men. This hypothesis also implies that the result will be more heterozygous women, i. e. carriers, than homozygous normal women.

The material is too small to enable us to determine whether this is the case or not.

The idea of a selective chromosome distribution is not new, a similar hypothesis having been used, e. g. by JULL (1924) in statistical study of the sex-ratio in poultry, where he considers he has shown that the age of the hen has a selective influence upon the distribution of the chromosomes at the maturation divisions.

It is evident that the same hypothesis may be valid, if the intersexuality is caused by an autosomal gene. It is also possible to form a hypothesis of selective fertilization. If *Y*-carrying spermatozoa more readily fertilize X_a than X_A eggs the result would be more intersexes than normal men. It is conceivable that the X_a eggs are most readily fertilized by a *Y* spermatozoon, the X_A eggs on the other hand by an X_A spermatozoon. This would result in more intersexes than normal men, but also in more homozygous than heterozygous women.

All these different assumptions, however, are mere conjectures, and cannot lead any further. We only wish to point out that if we do not accept the triploidy hypothesis, which seems rather improbable, the frequencies of Table 2 compel us to accept one or other of the auxiliary hypotheses. Which of those suggested here is the most likely one cannot be decided with the aid of the material available.

VII. A DISCUSSION OF THE DEVELOPMENTAL PHYSIOLOGY.

The data presented have not revealed any facts, supporting one or the other of the existing hypotheses concerning the developmental physiology, and our discussion of these questions may be rather brief. It seems desirable, however, to bring the results of section VI in accordance with known facts regarding the ontogeny and physiology of the sex characters.

The development of the sexual gland of the vertebrates is quite different in both sexes. In both of them sex-cords are proliferated from the germinal epithelium. If the embryonic gland is to develop into an ovary the first generation of these sex-cords, the primary sex-cords, are developed into the medulla of the ovary, and a second generation of sex-cords, the secondary ones, give rise to the cortex of the ovary, in which the eggs develop. When the embryonic gland develops into a testis the sex-cords give rise to the seminiferous tubules, whereas a cortex of the testis is known only in certain urodela

(*Amblystoma*; BURNS, 1931). It follows that, e. g. among the mammalia, the testis is morphologically homologous to the medulla of the ovary, whereas the cortex of the ovary has no homologous part in the testis. The homology, however, is not only morphological, but the medulla of the ovary is a potential testis, which under certain circumstances may develop into a real one. This is the case in the »freemartin», which is genetically a female calf, but in which the primary sex-cords develop — under the influence of the hormones of the male twin — not into a medulla but into testicular tissue, without the functional cells (CHAPIN, 1917; WILLIER, 1921). Several other cases of transformation of a female in a male direction are known among the vertebrates, the only known case of the reverse transformation — from male to female — being the one recorded by BURNS (1931) in *Amblystoma tigrinum*. But in this case the testis had a small cortex, i. e. some tissue homologous to the ovarian cortex.

Upon the above facts GOLDSCHMIDT bases his statement that all intersexes in Man and other mammalia must be female intersexes. According to his own definition an intersex is a sex-mosaic in time, i. e. an individual in which the sex development from the beginning of the ontogenese until a certain time — the turning-point — goes in the direction of one sex, after the turning-point in the opposite direction. The intersex is female, if it starts as a female, male if the male phase comes first. Another main group, according to GOLDSCHMIDT, comprises the gynandromorphs, which are defined as sex-mosaics in space, i. e. individuals, parts of which develop into female, simultaneously as the other parts develop into male. In supplementary reports (e. g. GOLDSCHMIDT, 1931) he adds that in the gynandromorphs the sexually different parts have different sets of chromosomes, whereas the chromosomes are the same in all the cells of an intersex. He points out that still other types of sex-mosaics may exist, but that all hitherto known and thoroughly investigated cases may be classified in one of the two categories just mentioned.

If, in defining a male intersex, we require that it must start its development as a male and, after the turning-point, continue as a female, and that even the sexual gland must partake in the transformation, it is fairly certain that such intersexes are non-existent in mammals. If, on the other hand, in defining an intersex, the stress is placed upon the disturbed balance between the substances *F* and *M*, which cause a development in female and male directions respectively, and not upon the occurrence of a turning-point, it is easily conceivable

that male intersexes may occur even among mammalia. From such a point of view CREW (1923), who accepts GOLDSCHMIDT's general views concerning sex-mosaics, has classified several sexually abnormal mammals (goat, swine, horse, cattle, sheep) described by himself, as male intersexes. A common characteristic of all his cases is the absence of ovarian tissue in their sexual glands. GOLDSCHMIDT (1931) groups CREW's cases with others, where ovarian tissue has been apparent, and considers all the cases to form a continuous series of intersexes. Knowing that a mammalian ovary may be transformed into a testis, but lacking the knowledge of the reverse process and, further, having from morphological view-points to consider the latter as rather improbable, GOLDSCHMIDT maintains, that CREW's cases are female intersexes.

With regard to the sex-mosaics in Man, GOLDSCHMIDT (1931) is of the same opinion. By bringing together the types described in the literature he forms a continuous series from nearly female to nearly male types with a large number of transitional types. The continuous series appears in the glands as well as in the secondary sex characters and GOLDSCHMIDT draws the same conclusion as in the case of other mammalia: all cases described represent female intersexes. In the present study we have brought certain earlier cases together with our own into one group, and in section V we have given the reasons for this grouping. All cases in this group — as far as it has been possible to ascertain — completely lack the ovarian tissue, but they have testes. Our main reason for considering them as male intersexes is, however, the frequencies in Table 2, discussed in section VI, which hardly allow any other conclusion than that the intersexes are genetical men.

We have also considered the possibility that the sex-mosaics may be triploid intersexes, but this hypothesis must be rejected, since such intersexes are abnormal in many other respects and have a low viability. But even if our intersexes were triploids it does not necessarily follow that the first stages in their development are female. DOBZHANSKY and BRIDGES (1928) have shown that the triploid *Drosophila* intersexes are to be considered as male intersexes, which, however, must not necessarily be true in the case of human triploids. It is even possible, as pointed out by BONNIER (1929), that all *Drosophilas*, even normal females, start their development as males. The studies of the development of the sexual glands in *Drosophila* females, made by KERKIS (1931), supported this hypothesis. In assuming analogous, but reverse, conditions in mammalia, we should assume that all em-

bryos, males, females and intersexes, start their development in a female direction, and that males and intersexes at a certain turning-point enter upon a male development. Such an assumption has some support in the morphological facts: the primary sex-cords develop similarly in both sexes, and then the turning-point for the normal males should be before the development of the secondary cords. Thus, if all normal males, i. e. *XY*-individuals, start their development as females it becomes quite natural to consider the sex-mosaics as female intersexes. The similar development of the primary sex-cords in both sexes is, however, a rather weak foundation for the assumption that the *XY*-individuals start as females. But if this assumption cannot be upheld, it is impossible to explain our sex-mosaics as female intersexes, and until further proofs are furnished, we do not consider that they ought to be classified as such.

As is evident from the above discussion, we do not offer a final developmental physiologic explanation of the type of intersex studied by us. We can, however, state that they are *XY*, and thus it seems probable that their internal genitals develop as in normal men. For some reason, which is probably the effect of a recessive sex-linked gene, no external male genitalia develop, the descensus is not complete and the testis produces ovarian hormones instead of normal testicular hormones, resulting in a general female exterior and female temperament. The occurrence in several cases of nasal bleedings and menstrual molinomina indicates, that the testes may secrete these hormones at regular intervals, just as normal ovaries. In discussing the »free-martin» GOLDSCHMIDT (1931) distinguishes between embryonic sexual hormones and final sexual hormones, and this distinction may to some extent explain the results. The embryonic hormones are taken to influence the glands themselves and the development of the derivatives of the Müllerian and Wolffian ducts, whereas the final hormones influence the secondary sex characters and the derivatives of sinus urogenitalis. If the main effect of the assumed sex-linked gene is to change the final testicular hormones in the direction of ovarian hormones the development of the intersexes described in the present paper would be at least partially clear, but the total absence of external male genitals requires further explanation.

SUMMARY.

1. A case of sex-mosaic in Man is described. An externally quite normal girl was operated upon for double inguinal hernia and was

found to have testes and other internal male genitals but no female internal genitals.

2. Similar sex-mosaics within the same family are described and a review is made of published cases, where several members of a family have shown a similar type of intersexuality.

3. The only simple genetical explanation fitting the frequency data obtained is that the intersexes in question are triploids (aneuploid trisomics). This explanation must, however, be rejected, since triploid intersexes, because of the strong disturbance in the chromosome-balance, are most probably abnormal in several anatomical characters and have a low viability (as is the case of the triploid intersexes in *Drosophila*), whereas the human intersexes in question are normal in all respects except the sex. Further, inasmuch as the frequencies deviate widely from those expected under the assumption that the intersexes are *XX*, but fit with the frequencies expected if they are *XY*, they are assumed to have the latter constitution.

4. Alternative genetic interpretations are given, among which the most probable one seems to be the assumption that the intersexes are due to a recessive sex-linked gene. Any genetic explanation, however, except the assumption of triploidy, implies the aid of one or the other of a number of auxiliary hypotheses, if the proportion between normal males and intersexes is to be explained.

5. The developmental physiology of the intersexes is discussed. Since they are *XY* it is considered very improbable that they are to be classified as female intersexes. The most probable explanation seems to be that they start development as normal males, but that the sex-linked recessive gene completely inhibits the development of external male genitals, more or less completely the descent and that it causes the hormones of the testes to change into ovarian hormones.

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THE EFFECTS OF THE MINUTE MUTATIONS OF DROSOPHILA MELANOGASTER ON DEVELOPMENTAL RATE

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INTRODUCTION.

IN recent discussions of the factors determining the specific characters of animals, the *rates* at which the processes of development occur have occupied a leading place. This is due in part to the discovery that one of the conditions necessary for normal development is the maintenance of an orderly rhythm of correlated growth processes in the embryo. When this is disturbed by external agents the parts of the embryo may be markedly altered from the normal. The alterations, as CHILD, STOCKARD and others have shown, appear chiefly in those parts which are in a sensitive state, often one of high metabolic activity, at the time when the disturbing agent is applied. Similar character alterations also appear in races of animals with mutant genes, and there is evidence that in some cases the mutant genes have altered the rates at which certain developmental processes occur and have thus altered the adult characters from their normal condition. Such effects have frequently been invoked in interpreting the results of experimental observations on the expression of mutant genes, for example GOLDSCHMIDT (1920), PLUNKETT (1926), FORD and HUXLEY (1927), WRIGHT (1934); and a general theory according to which the genes determine the characters of the organism through controlling the rates of developmental processes has been developed by GOLDSCHMIDT (1927).

Although such a general assumption is consistent with what we know of the gene and development, the evidence regarding the detailed mechanisms by which development is affected is largely indirect and rests on inferences from the conditions of mutated characters at the end of or (more rarely) during development. A somewhat different approach to the problem has been made possible by a peculiarity of many mutant genes; namely that their effects seem to be less stable and more easily altered by external conditions than the effects of their wild type allelomorphs. It may thus be determined experimentally

what effects on development are brought about by agents which influence developmental rate, in the same way as previously done by experimental morphologists, but with the additional advantage of using genetically known materials, and of correlating at the same time the effects of the mutant gene with the amount and kind of external change experimentally induced. A specific example of the results obtained by this method is the demonstration by ZELENY, KRAFFKA, DRIVER, HERSH and others that an orderly relationship exists between the size of the eye in the Bar mutant of *Drosophila melanogaster* and the temperature obtaining during a short period of larval life. The relationships show that the size of the Bar eye is determined during a short critical period in larval life and it has been assumed that the Bar gene influences the rate of some process during this period which is concerned with the reduction or destruction of eye-forming material.

The occurrence in *Drosophila* of mutant races in which the rate of development differs markedly from that of the wild type suggests that such mutations might be substituted for external agents as a means of testing directly the effects of changes in developmental rate upon the expression of other mutant genes.

This method consists in brief of measuring the effects in combination of two mutant conditions one of which has an effect on the rate of development which can be observed directly, the other an effect on some readily observed and measured structure. Two examples of the use of this interaction method have been published recently. MARGOLIS (1935 a and b) showed that the vestigial mutant of *Drosophila melanogaster* causes a slight but significant extension of the larval period and a slight but significant decrease in the number of facets in the Bar eye. The relationship obtained over a wide range of experimental temperatures. MARGOLIS concluded that the vestigial gene modified Bar through effecting an extension of the critical (temperature-effective) period for Bar in late larval life, permitting the facet-destroying process in Bar-vestigial to act for a longer time. DUNN and COYNE (1935) in a preliminary report showed that several of the Minute mutants of *Drosophila melanogaster* delay larval development and reduce the size of the eye of the Lobe mutant form. There was an indication that the amount of eye-reduction was roughly proportional to the amount of larval delay brought about by the different Minute mutations. They assumed that the modification of eye size by the Minutes was due to a general reduction in developmental rate during a period in embryogeny when the eye anlage was undergoing rapid growth.

Although neither demonstration was conclusive, both indicate the usefulness of mutants affecting larval development as tools in analysing the general problem. In this paper we report observations on some members of one such group of mutants, the Minute mutants of *Drosophila melanogaster*. The observations show that each mutant studied has a characteristic effect on the duration of larval life, probably on the speed of growth during this period. Later papers will deal with the effects of these mutants on the expression of mutant eye characters.

THE MINUTE MUTATIONS.

A great many different Minute mutants whose loci are scattered through all four chromosomes have been found in *Drosophila* since the discovery of the first mutant of this type by BRIDGES in 1919. All affect the bristles similarly, but in varying degrees; all except one are dominant and lethal when homozygous; several have been shown to be section deficiencies; and many are known to hatch later than non-Minute sibs in the same culture. SCHULTZ (1929) who has made a careful study of the effects of twenty-five Minutes both alone and in combination with each other and with other genes has characterized them as follows: »Many other characters than the bristles are affected in these mutants. The eyes are larger and rougher than normal; the wings are blunt in shape, sometimes beaded. Their venation is slightly plexus-like, and the fifth vein may stop short of the wing margin. Minute flies have weak legs and a somewhat pale body color; the aristae may be reduced, the abdominal scelerites irregular. The postvertical bristles of the head are sometimes missing, and other bristles (notably the alars) are occasionally duplicated. The total body size may be smaller than normal. Females are likely to be sterile and are generally weaker than males. Most striking of all the characters of the Minutes, however, are the *retardation of development* and the elimination of chromosomes in the somatic cells».

Of the six Minutes used in the following experiments only two (*Mz* and *Ml*) were found to have eyes appreciably larger and rougher than normal; the eyes of the others only occasionally showed a slight roughness. Otherwise SCHULTZ's remarks also apply to the Minutes used by us. SCHULTZ found that the bristle effects of different Minutes were not cumulative in combination and concluded from this that each Minute influences a different primary reaction. He made a few experiments, of a preliminary nature, to test whether this conclusion

applied also to the effects of Minutes on retardation of development. The data and methods are not reported in detail but apparently refer to the duration of the egg-larval period in Minutes and comparable non-Minutes in the same cultures and these constitute the only data on this stage apart from those reported in the present paper. The differences in days, between average time of development (temperature not specified) of non-Minute sibs and *Mh*, *Ml*, and *Mh Ml* respectively as calculated from SCHULTZ's data are as follows:

Exp.	*Ml*	*Mh*	*Ml Mh*
A	2,7	0,6	1,1
B	0,9	1,2	1,0
C	1,8	2,0	1,8
D	1,5	0,7	1,5

The differences were all plus in sign, that is all Minutes developed more slowly than non-Minutes. Although *Ml* and *Mh* did not show constant differences from each other or from wild type it is clear that their effects on development were not cumulative.

It was therefore conceivable that different Minutes might exercise different effects on the rate of development and thus provide means for analysing the relations between speed of development and the effects of mutant genes. Our first experiments then were designed to measure the degree of retardation and the period of development affected by several Minutes.

The Minutes chosen for the preliminary experiments were those in stock at the Department of Genetics, Cold Spring Harbor, N. Y. where the first observations were made during the summer of 1934. Later, one further Minute (*Ml*) was added to these. The Minutes used, all of which have readily recognizable effects on the bristles, were as follows:

Minute	*Locus*	*Cultured as*	*Remarks*
Mz	II, 13,0	*Mz b*/*Cy*	
Ml^2	II, 101,0	*Ml*^{2}/*Cy L*^{4} *sp*^{2}	allel of *Ml* (not a deficiency)
Ml	II, 99—101	*Ml*/*Cy*	deficiency for *px* (100,5) and *arc* (99,2)
Mh	III, 40,4 ±	*se Mh H*/LVM	
M33j	III, 40,4 ±	*M33j*/+	deficiency for *Ly*
Mw	III, 80	*Mw*/C_3 ca	

In the first experiments wild type females were mated soon after emergence with males of the Minute to be tested. The mated females were aged for 2—3 days; then were isolated in individual vials in each of which was a piece of colored blotting paper infused with banana-agar-yeast food mixture supported on a microscope slide. Each female was allowed to lay for 8—10 hours, the eggs being deposited on the food-covered paper. The slide with paper and eggs was then removed, the number of eggs counted, and the slide with eggs was transferred to a vial with fresh banana-agar food mixture, into which the larvae crawled as soon as they hatched. Vials were kept at 25° C. In the first experiments all of the eggs on the slide were transferred to the food vial. This resulted in unequal numbers of larvae in the different vials, in some of which the larvae were crowded and grew more slowly. After 72 hours the slides were removed and the unhatched eggs left on it were counted. The larvae completed their growth in the food vials and pupated on the sides of the vial. The vials were examined every 24 hours and each pupa formed during the preceding 24 hours was marked with a distinctive sign indicating the time of pupation. These marks were made on the glass above each pupa with a diamond pencil. As the food became drier some larvae pupated in the food. These were removed with a needle, fastened to the glass, and marked. A few errors may have occurred in this process since a few fresh pupae in the food may have been overlooked and not removed until twelve hours later, but with increasing practice and the use of moist food such errors were few and had no important effects on the results. When eclosion began observations were made every twelve hours. All flies which had emerged during the preceding 12 hour period were classified as Minute or non-Minute, and the empty pupal cases from which they had emerged were recorded and removed. The records thus obtained show the length to within about 12 hours of the egg-larval and pupal periods for each individual and allow the detection of any marked differences in the length of these periods as between Minute and non-Minute flies.

According to LOEB and NORTHROP (1917) the egg-larval period of *D. melanogaster* requires about 8 days at 20° C, 5 days at 25° C and 4 days at 27,5° C. The duration of pupal life at these temperatures is about 6,3 days, 4,2 days and 3,2 days respectively. CHEN (1929) estimated the length of the larval period (after emergence from the egg) at 25° C as 4 days and the pupal period as 4 days. DOBZHANSKY and DUNCAN (1933) found the duration of the larval period at 27° C to be between 80 and 100 hours; the pupal period about 90—110 hours.

The duration of these periods is very sensitive to variations in the environment; in addition to temperature, the amount and kind of food (yeast) available, the number of larvae competing for food, the humidity etc., play important rôles; so that it is necessary that the Minutes be compared only with non-Minutes from the *same cultures*. Even here fluctuations of random sampling and of food conditions in the same lot of cultures are sufficiently difficult to control to make it advisable to have experience from repeated experiments.

MINUTE DELAY EXPERIMENT 1.

These preliminary tests involved *Mw*, *Ml*^{2}, *Mz*, *Mh* and *M33j*. The results, although not as significant as those of later experiments because of failure to prevent crowding of the larvae, are nevertheless

TABLE 1. *Comparison of duration of egg-larval, pupal and total development periods in various Minute mutants and their non-Minute sibs in the same cultures. Crowded cultures at 25° C.*

	No.	Average egg-larval period. Days	Average pupal period. Days	Average period egg to imago. Days
Mw	261	8,9	4,6	13,5
+	292	5,6	4,4	10,0
Difference		3,3	0,2	3,5
Ml^2	105	6,8	4,0	10,8
+	74	6,1	4,0	10,1
Difference		0,7	0,0	0,7
Mh	76	7,1	4,6	11,7
+	113	5,5	4,5	10,0
Difference		1,6	0,1	1,7
Mz	32	6,5	4,6	11,1
+	47	5,3	4,4	9,7
Difference		1,2	0,2	1,4
M33j	184	8,9	4,2	13,1
+	238	5,9	4,7	10,6
Difference		3,0	−0,5	2,5

sufficient to show that each Minute delayed *larval* development as compared with non-Minute sibs from about one to three days. In the *Mw* test the larvae which pupated on the 4th to 6th days were non-

Minute; of those pupating on the 7th, 8th and 9th days some were Minute and some non-Minute; those pupating after the 9th day were Minute (one exception). Similarly, nearly all flies which eclosed on the 8th—10th days and nearly all of those on the 11th day were non-Minute; on the 12th and 13th day appeared Minutes and non-Minutes, while all which eclosed after the 13th day were Minute. It is clear from Table 1 that the period of pupation is slightly over four days for both Minute and non-Minute flies and that the retardation of the Minutes is thus confined to the egg-larval period. The average durations of egg-larval and pupal periods in Minute and non-Minute sibs are shown in Table 1. That conditions in the different experiments, except the last *M33j* test, were reasonably comparable is shown by the very similar periods of total development of the non-Minute flies, 10,0, 10,1, 10,0 and 9,7 days. In the *M33j* test this period was 10,6 days, and there was also a difference in the duration of the pupal period as between Minute and non-Minute. In this experiment one observation period was missed and the difference in duration of larval period is probably too large by not more than half a day. In all of these experiments the Minute flies were taken directly from stock so that the different Minutes are associated with genic backgrounds which may not be identical, although later experiments showed that when they were made identical similar results were obtained. These tests however are sufficiently accurate to reveal the general fact that each of these five Minutes develops more slowly than non-Minute sibs in the same cultures; that the delay is confined to the larval stage, and that different Minutes probably cause different degrees of delay. The order of their delay effects in these experiments was *Mw*, *M33j*, *Mh* = *Mz*, *Ml*^{2}.

Mw, *M33j* and *Ml*^{2} were chosen as representing marked differences in the degree of larval delay and were studied more carefully. The observations from this point were carried out at the Anatomical Institute of the University, Oslo. In the repetition of the above experiments the number of eggs placed in each vial was the same (20 or less) and pupation and eclosion were both recorded at 12 hour intervals. In all subsequent experiments, freshly made banana-agar culture medium was used, containing a small amount of Nipagin-M (0,15 %) to reduce the growth of molds. Previous tests with this mixture had showed that it produced normal growth, and that the amount of Nipagin-M used did not delay the growth of the larvae. Dry Magic Yeast was used for seeding the cultures, but later fresh baker's yeast was found to give larger yields and was used as a standard. The times of development

were calculated from the midpoints of the periods between observations. Thus the age of the eggs laid between 6 P.M. and 9 A.M. was reckoned from 2 A.M.; pupae found at 9 A.M. were assumed to have been formed at 2 A.M.; those found at 6 P.M. to have been formed at 2 P.M.; similarly for the time of eclosion. In most experiments the temperature was held at 25 ± 1° C; one test was run at a lower and one at a higher temperature. In the later experiments the Minutes used were from the 3rd—5th generations of successive backcrosses to the same wild type stock.

MINUTE DELAY EXPERIMENT 2.

The effects of *Mw* and *Ml*^{2} were compared at 21 ± 2° C. Wild type females were crossed with *Mw*/C_3 ca and with *Ml*^{2}/*Cy L*^{4} *sp*^{2} males from stock. Eggs from one ten hour laying period were used, and observations made as above. The results are shown in Table 2.

TABLE 2.

	No.	Egg-larval period. Days	Pupal period. Days	Total development. Days
Mw	30	9,3	6,1	15,4
C_3 ca	37	6,5	5,8	12,3
Difference		2,8	0,3	3,1
Ml^2	47	7,2	5,8	13,0
Cy L^4	34	6,3	5,7	12,0
Difference		0,9	0,1	1,0

About the same amount of delay was found as in Exp. 1 and again it was confined to the larval stage. The presence of *L*^{4} and *Cy* in the non-Minutes did not appreciably affect the speed of larval development since the larvae pupated in 6,3 days as compared to 6,5 for wild type. The whole period of development was about two days longer than in Exp. 1 due to the lower temperature, the increase occurring in the pupal stage. Absence of larval delay due to crowding compensated for the delay due to lower temperature, the larval periods of *Mw* and *Ml*^{2} and non-Minutes being similar respectively to those in Exp. 1. The different conditions in Exp.1 and 2 did not, however, affect the relative differences between *Mw* and *Ml*^{2}. *Mw* produced from three to four times as much larval delay as *Ml*^{2}. In this experiment the larval and pupal

periods of *Mw* and + showed almost no overlapping variability (Table 3) while *Ml*[2] and + showed considerable overlapping.

TABLE 3. *Comparison of lengths of larval and pupal periods of Mw and wild type sibs from same cultures, at 22° C. Data from individually marked pupae of Exp. 2. + offspring in Roman; Mw in bold face.*

Time of pupation	Time of Eclosion in Days from Egg-laying													Totals pupated + *Mw*	
	11	11,5	12	12,5	13	13,5	14	14,5	15	15,5	16	16,5	17		
5,5	1	4												5	
6	2	3	6	2										13	
6,5		1	1	7										9	
7					2									2	
7,5					1	5	1	1						7	**1**
8							1	1						1	**1**
8,5							1	1	1						**3**
9								1	8	3					**12**
9,5									1	1	5				**7**
10												2			**2**
10,5												1			**1**
11												1	1		**2**
11,5												1			**1**
Total +	3	8	7	9	3	5	2							37	
Eclosed *Mw*							1	4	10	4	5	5	1		**30**

DELAY EXPERIMENT 3.

In this experiment, planned to measure more exactly the relative delays brought about by *Mw* and *Ml*[2], 280 eggs from + ♀ × *Mw*/+ ♂ (*Mw* extracted from two backcrosses to the standard + stock) and an equal number from + ♀ × *Ml*[2]/*Cy L*[4] *sp*[2] ♂, all laid within a seven hour period, were divided into lots of 20 eggs each and placed for development in large vials (11 × 3 cm) with equal amounts of banana-agar food, in an incubator adjusted to 25° C. In many of the vials the food mixture on the egg slides became too dry and many of the eggs failed to hatch. When the results from these vials were discarded the numbers remaining were rather small, but consistent. The results are shown in Table 4.

The difference in the distributions of pupation times is shown in Table 5.

The lengths of pupal and larval periods were shorter at 25° than at 21° in Exp. 2, but the *relative* larval delays brought about by *Mw* and

Ml^2 were similar. *Mw* caused about four times as much delay as *Ml*^{2}. The order of effect of the two Minutes is thus similar under the three different sets of conditions.

TABLE 4.

	No.	Egg-larval period. Days	Pupal period. Days	Total development. Days
Mw	53	6,5	4,0	10,5
+	44	4,7	3,8	8,5
Difference		1,8	0,2	2,0
Ml^2	24	5,0	4,1	9,1
Cy L^4	25	4,6	4,0	8,6
Difference		0,4	0,1	0,5

TABLE 5.

Time of pupation in days from egg laying	Number of pupae			
	Mw test		*Ml*^{2} test	
	+	*Mw*	*Cy L*^{4}	*Ml*^{2}
4,0	9		5	
4,5	10		13	8
5,0	24		6	10
5,5	1	2	1	5
6,0		18		1
6,5		18		
7,0		13		
7,5		1		
8,0		1		
Totals	44	53	25	24
Average time	4,7	6,5	4,6	5,0

In order to increase the number of observations on the *Ml*^{2} effect, this experiment was immediately repeated with ten large tubes of 40 eggs each from + ♀ × *Ml*^{2}/*Cy L*^{4} *sp*^{2} ♂, all laid within the same 12 hour period and reared at 25° C. This experiment was planned also to reveal whether any of the delay associated with a Minute occurred in pre-larval development, that is, before the hatching of the egg. The eggs on each slide were accordingly divided into two lots, those hatching early and those hatching late. This was accomplished by examining the slides when about half the eggs were hatched. The larvae already

hatched were transferred to the food and the slide containing that half of the eggs which had not hatched was transferred to a new vial. In this case development in general was slower than usual and half of the eggs had hatched only after 32 hours. The early hatching eggs produced 115 *Ml*^{2} and 81 non-Minute (*Cy L*^{4}) flies, showing that the delay associated with *Ml*^{2} probably does not take place in the egg stage. Most of the remaining half of the eggs failed to hatch, possibly because of the handling and cooling to which they were subjected when examined just before they were to hatch. The larvae from early hatching eggs grew very slowly and early growth appeared to be impeded, probably by a defect in the food which affected other cultures prepared at this time. The average length of the egg-larval period was thus longer than usual in both Minute and non-Minute. The Minutes pupated between the 5th and 9th day with an average larval period of 6,8 days; the non-Minutes pupated between the 5th and 12th days with an average larval period of 7,5 days, that is, 0,7 days *longer* than the Minutes. This is the reverse of the order found in all other experiments. Apparently the non-Minutes were more delayed by unfavourable conditions than the Minutes, and the excess of Minutes may mean that the Minutes also suffered less mortality. This is in line with the general experience that Minutes survive better at low temperature which retards the rate of larval development. Other evidence (Exp. 2, 3) shows that *Cy L*^{4} has probably no marked effect on larval growth. The pupation periods were not affected by the culture conditions: 4,2 days for *Ml*^{2}; 4,1 days for non-Minute.

Because of the probable defect in the food the results cannot be used for comparison with other measurements of Minute delay. The reversal of the Minute effect shows at any rate that the effect of a Minute depends in part upon the general rate of development as determined by other conditions.

EXPERIMENT 4.

To test the effect of *M33j* on larval growth, + ♀ were mated with *M33j*/+ ♂ from the second backcross to + stock; eggs from one twelve-hour period were saved and reared in large vials, 20 to a vial at 25° C. There was a rather high mortality of eggs, and some delay in the beginning of pupation. The results are shown in Table 6.

The *M33j* larvae took two days longer to pupate than their wild type sibs and the delay was confined to the larval stage. The lengths of the larval periods and of total development for the non-Minutes were

TABLE 6.

	No.	Egg larval period. Days	Pupal period. Days	Total development. Days
M33j	55	7,9	4,0	11,9
+	67	5,9	4,0	9,9
Difference		2,0	0,0	2,0

about half a day longer than in Exp. 3 at the same temperature, so that conditions were not perfect; but the experiment serves to show that *M33j* delays larval development considerably.

EXPERIMENT 5.

A fifth Minute (*Mz*) was tested simultaneously with a repetition of the *M33j* test. In each case + ♀ were crossed with Minute ♂ which had been backcrossed to the standard + stock. Eggs were collected over a four-hour laying period. Twenty eggs were placed in each vial in an incubator. The actual temperature varied from 21—23° C; other conditions and methods were as in Exp. 4. In this experiment there was practically no overlapping between the + and Minute classes either in time of pupation or of eclosion. Non-Minute larvae pupated within about 36 hours after pupation began, while the Minute larvae did not begin to pupate until 12 to 36 hours later. Nearly all Minutes had pupated at the end of another 36 hours. In this experiment as in others there were minor variations between the different vials. These results are given in detail in Table 7 as illustrative of the data from such experiments.

From this table it is clear that within the same vial the + larvae have all pupated (with one exception in vial 11 *Mz* test) before the Minute larvae begin to pupate. Usually there is a period of from 12 to 24 hours when no new pupae are formed. This falls for *Mz* and *M33j* always in the third or fourth 12-hour period after the beginning of pupation. Where the beginning of pupation is delayed as in vial 5 *M33j* test, this gap and the beginning of pupation of Minutes are also delayed. The variations in pupation times in different vials are probably due chiefly to chance, but there may be minor differences in the amount of yeast available or in humidity, etc.

The average times of pupation for +, *M33j* and *Mz* larvae as calculated from Table 7 together with average times of eclosion and duration of pupal life are given in Table 8.

TABLE 7. *Records of times of pupation of larvae in Exp. 5. Eggs laid by + ♀ fertilized by M33j/+ ♂ and by Mz/+ ♂. Eggs laid 6—10 PM 1/14/35. Heavy bars in columns mark the time after which all pupae produced only Minutes.*

Days from egg	Number of new pupae on	+ × *M33j* + Vial numbers									Totals + *M33j*		+ × *Mz*/+ Vial numbers											Totals + *Mz*	
		1	2	3	4	5	6	7	8	9			1	2	3	4	5	6	7	8	9	10	11	12		
5 1/4	1/20 9 A	1		2	1		1	6	5	1	17		3	3		9	6			4	4	9	1	8	47	
5 3/4	6 P	4	6	2	3		3	1	4	3	26		4	6	4		5	3	6	6	3	2	4	1	44	
6 1/4	1/21 10 A	2	3	1		3	2			1	12				1			2	3	1			4		11	1
6 3/4	7 P			7		4		1	5		4	13	2			3	2		1	3			1	1	1	11
7 1/4	1/22 10 A	8	5	2	10		7	7	3	6		48	4	4	9	7	3	5	8	4	4	6	7	10		71
7 3/4	7 P	1	3	2	4	4	4		3	1		22	1	2	1	1	3	2	.3	1		1				15
8 1/4	1/23 9 A		3	1	1	6						11						1		1	1					3
	6 P					1						1		1							1					2
	1/24 9 A								1			1		1	1											2
	Totals	16	20	17	19	18	17	15	21	12	59	96	14	17	16	20	19	13	21	20	13	18	17	20	103	105

TABLE 8.

	No.	Egg larval period. Days	Pupal period. Days	Total development. Days
M33j	96	7,5	5,5	13,0
+	59	5,8	4,6	10,4
Difference		1,7	0,9	2,6
Mz	87	7,3	5,1	12,4
+	96	5,6	5,0	10,6
Difference		1,7	0,1	1,8

Both *Mz* and *M33j* cause about the same delay in larval development. The results for the pupation periods for both *M33j* and + are exceptional, the period for *M33j* being longer than usual and that for the + sibs being shorter. The difference of nearly one day between these two appears to be rather large for a random deviation, yet no other explanation is apparent. In view of the other experiments it is unlikely that *M33j* is responsible for any delay in the pupal stage.

EXPERIMENT 6.

This experiment was designed to afford a direct comparison of the relative effects of *Mw*, *M33j* and *Ml* on larval growth, to test their modifying effects on Lobe eye and to detect any marked effect on speed of development of the Lobe gene, which was used in the interaction experiments to be reported later. Females *L*/+ from the third backcross of *L*/+ by wild type (Florida stock) were crossed with males *Mw*/+, *M33j*/+ and *Ml*/+ respectively, all derived from backcrosses to the same + stock. In this experiment the mated females were kept for six days in small vials with little food, a condition which is known to cause retention of eggs and to produce rapid hatching of eggs laid subsequently. They were then transferred to vials with yeasted paper and allowed to lay for six hours. The eggs were counted and twenty were placed in each large food vial for rearing where they were observed to hatch very quickly. The vials were placed in an incubator adjusted to 25°, but during the first six days of the experiment the thermostat stuck and temperatures of 26—28° C were recorded on three days. Development under these conditions was very rapid, the first pupae appearing in 3 $^1/_2$ days and the first imagos in 7 days. The results of the experiment are shown in Tables 9 and 10.

TABLE 9. *Distribution of times at pupation (by hours) in Exp. 6.*

Hours from egg	+	*Ml*	+	*Mw*	+	*M33j*
85	4		26		11	
93	22		15		15	
105	6		2	3	12	2
117		7		15		10
129		20		10		20
141		6		4		7
153		13		2		1
165		3				
n	32	49	43	34	38	40

TABLE 10.

	No.	Egg larval period. Days	Pupal period. Days	Total development. Days
Ml	32	5,67	3,75	9,42
+	49	3,91	3,46	7,37
Difference		1,76	0,29	2,05
Mw	34	5,67	3,87	9,54
+	43	4,13	3,58	7,71
Difference		1,54	0,29	1,83
M33j	40	5,29	3,83	9,12
+	38	3,91	3,64	7,55
Difference		1,38	0,19	1,57

In all three tests, the Minute larvae developed more slowly than the non-Minutes in the same culture. *Ml* and *Mw* larvae required the longest time for growth, each pupating in about 5 $^{3}/_{4}$ days (the extreme range in time before pupation was 5—7 days for each type). The effect of these two Minutes on larval development is apparently very similar, the smaller difference between *Mw* and its control being due to the slightly slower development of the non-Minute larvae in these cultures. The ranges and averages both show that *M33j* causes less retardation. The small differences in the pupal periods are probably significant, and may be due to the slight fall in temperature from about 27° to 25° during the pupal period which would affect the later pupating Minutes more than the earlier non-Minute pupae.

A feature of the experiment was the great rapidity with which all stages of development took place. The total average time from egg to imago was only 7,5 days for the 130 non-Minute offspring, which corresponds with the times found by LOEB and NORTHROP and DOBZHANSKY and DUNCAN at 27° and 27,5°. Most of the acceleration occurred in the egg-larval stage, the length of the pupal stage being only a little less than normal. The increase in the rate of larval development did not change the relative durations of the periods as between Minute and non-Minute.

In this experiment four types of offspring appeared: wild type, Lobe, Minute and Minute Lobe. The average eclosion times of these classes were 7,7, 7,7, 9,3 and 9,4 days respectively. The order of pupation and eclosion is the same for Lobe and non-Lobe. Lobe therefore has probably no marked effect on the length of larval development.

EXPERIMENT 7.

A further test of the relative order of the delay associated with *Mw*, *Ml* and *Mz*, and of possible effects on Lobe^2 (the most extreme Lobe allelomorph) on speed of development was made using the same methods as in Exp. 6, except that individual pupae were not marked and only differences in the total time of development were measured. Females $L^2/+$ from the 5th backcross to + Florida stock were mated with males $Mw/+$, $Ml/+$ or $Mz/+$, also from 5th backcross to + Florida stock. After five days eggs were collected from a three-hour laying period and incubated at 25° C, 20 eggs per large food vial. The vials from $L^2/+ \times Mz/+$ gave too few offspring to be significant. The average times of development are shown in Table 11.

The delay associated with *Mw* appears to be slightly greater than with *Ml*. Lobe^2 flies did not differ from wild type sibs in speed of development. No Minute Lobe^2 combinations hatched, so the total number of Minutes is only half of that expected. The lethal interaction of L^2 and Minute is discussed elsewhere.

In Experiments 5, 6 and 7 the sexes of all offspring were recorded. Among the non-Minute offspring the females pupated and eclosed a little more rapidly than the males, but the difference was small, about $^1/_4$ day for duration of larval life and $^1/_2$ day for total development, due chiefly to the fact that females begin to pupate and eclose first, although they continue to do so over as long a period as the males. Among the Minutes, there was no sex difference in the duration of larval life (in Exp. 5 and 6) and only a small difference in total time of development,

the females of *Mw*, *M33j* and *Mz* eclosing on the average of about $^1/_4$ day earlier than the males. In Exp. 7 *Ml* females eclosed later than *Ml* males by about a half day. This may indicate that the females, which normally develop more rapidly, are somewhat more retarded by Minutes. This may contribute to the greater mortality among females which is associated with Minutes with extreme retarding effects.

TABLE 11.

	No.	Total development. Days
Mw	57	12,15
L^2 and +	113	9,46
Difference		2,69
Ml	36	11,54
L^2 and +	94	9,31
Difference		2,23
All L^2	91	9,33
All +	116	9,36

ORDER OF RETARDING EFFECT OF THE MINUTE MUTANTS.

In considering the evidence on the retarding effects of the six Minutes studied, the first facts of importance are that under normal culture conditions all of them consistently take *longer* to develop than non-Minute sibs in the same cultures; (2) the longer development is in all cases due to their longer larval periods. There are some differences in the lengths of pupal periods but these are all very small in proportion to the differences in the larval periods. Generally, where there is a difference, the pupal period of the Minute is slightly longer than that of the comparable non-Minute. Except in the case of *M33j*, none of these differences exceeds 0,3 days, and in the four tests of *M33j*, one experiment showed no difference, one a difference of — 0,2 and in two others the differences were of opposite sign. Although the data do not exclude the possibility of a slight retarding effect of Minutes on the pupal stage, they show that the significant differences are certainly those which occur in the larval stage.

The estimation of the relative amount of larval retardation as-

sociated with the various Minutes is made difficult by the different conditions surrounding the several tests and by the variations in time of pupation of larvae of very similar age and genetic constitution. Variations of the latter sort have been reduced by using only eggs laid within a few hours, but there are always uncontrollable variations in the stage of development of the eggs when laid. From the same female, some eggs hatch very quickly, others only after 24 hours or more. These variations have been reduced by our practice of ageing mothers before eggs are saved, but they have been a constant and annoying source of variation. These variations probably affect Minutes and non-Minutes alike, but they indicate that differences calculated from such variable data are only significant when repeated experiments give similar results.

The cultural differences consisted chiefly of (1) degree of crowding which was eliminated as a variable in all experiments after the first; (2) differences in temperature; (3) differences in the character of the culture medium. The effects of the last two on developmental rate may be minimized by comparing not the absolute differences in time required by Minute and non-Minute in different experiments, but these differences as *proportions* of the time required by the wild type. Thus *M33j* was tested in three experiments (4, 5, 6), after crowding was eliminated. It increased the duration of larval development in these tests by 2,0, 1,7 and 1,4 days respectively, but the larval periods of the non-Minute controls were 5,9, 5,8 and 3,9 days. The *proportional* increases over the non-Minutes due to *M33j* were thus 34, 29 and 36 per cent respectively. When the data are examined in this way, it is found that in spite of the variations between experiments, the several Minutes tested always show the same order of effect. *Mw* always produces the greatest proportional increase in length of larval period followed in order by *M33j*, *Mz* and Ml^2.

The degrees by which these Minutes extend the larval period are about as 40 : 33 : 30 : 12. The differences between *Mw* and Ml^2 in this respect are always very marked, *Mw* having a considerable retarding effect, Ml^2 a consistently small one in the proportion of about 3 : 1. *M33j* and *Mz* have about the same effect, always less than *Mw* and always a little over twice as great as Ml^2. *M33j* and *Mw* have been directly compared in only two experiments, and the proportion between their effects cannot be regarded as well established yet. *Ml* has been tested only twice. When compared directly with *Mw* and *M33j*, it has a retarding effect similar to that of *Mw* and greater than that of *M33j*.

A second test showed it to have a marked effect, probably slightly less than that of *Mw*. *Mh* was tested only in Experiment 1, in which its effects fell between those of *M33j* and *Mz*, but in the absence of confirmation this may be neglected. *Mh* has not been used in the interaction experiments to be reported later.

The significant conclusion from the above is that in these Minutes we have agencies under genetic control by which the larval development may be retarded by various degrees. They thus provide excellent tools for use in approaching the problem — What influence do variations in the rate of larval development have upon the developmental effects of mutant genes?

One limitation to the usefulness of the Minute mutations for this purpose is our present ignorance concerning how their effect on developmental rate is exerted and especially whether they reduce the rate of growth at some specific period or extend the length of the growth period as in larvae reared at low temperature or in the mutant giant (GABRITSCHEVSKY and BRIDGES, 1928).

In both of the latter cases extension of the larval period results in increased body size; in the Minutes examined by us, body size in spite of the longer larval feeding period is generally smaller than in non-Minute sibs. Since Minutes take a longer time to attain less size, their growth must be less efficient and probably slower than in non-Minutes. It thus seems proper to refer to their effects as »retarding» rather than merely as »extending» larval growth.

This effect probably occurs in the active larval (post-embryonic) period, since preliminary evidence indicates that Minutes hatch from the egg at about the same time as normal sibs. The possibilities remain, either that the lower growth rate is spread over the whole larval period or that growth which has begun at a normal rate is later reduced by the action of a Minute. The question cannot be settled from the present evidence, but several considerations make the latter alternative more probable. The rate of growth of the earliest stages is probably influenced largely by the constitution of the egg and the environment; the innate growth efficiency of the larva would require a certain time to express itself. This would be true especially if the Minute effect should prove to be due to deficiency, i. e. to haploidy for certain normal genes, for in that case there should arise an accumulation of products normally converted by the interaction of all the genes, and any depressing effect of such products on metabolism would occur only after a certain concentration of them was reached. Several Minutes have

proved to be section deficiencies, but one at least (Ml^2) apparently is not (MORGAN, BRIDGES and SCHULTZ, 1934), so the argument cannot be generalized. Minutes generally survive better at lower temperatures, and males, which have a slightly lower growth rate than females, are less adversely affected by Minutes than are females; moreover in one experiment delay in early larval growth due to culture conditions affected survivals of Minutes less than non-Minutes. These facts are consistent with the assumption that the Minute effect on growth supervenes in later stages and consists essentially in a *change of rate*. Its effect on viability and on characters sensitive to changes in growth rate should thus be less when previous development had been slow, greater when previous development had been rapid. A similar assumption, that Minutes are effective in modifying eye characters only when they impose a marked change in developmental rate, emerges from the data to be reported in later papers. The mechanism by which Minutes affect development is, however, a special problem which can be attacked directly, and for the present we may merely note that its essential feature seems to be to bring about a *relative* difference in growth rate in successive periods of larval life.

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GENSTUDIEN AN PISUM SATIVUM

II. GESTREIFTE SAMENSCHALE UND IHRE VERERBUNG

VON *HERBERT LAMPRECHT*

SAATZUCHTANSTALT WEIBULLSHOLM, LANDSKRONA

(With a summary in English)

VOR einigen Jahren erhielt ich im Tauschwege eine Anzahl von Samenproben von *Pisum sativum*, unter denen sich eine durch eine mir bis dahin unbekannte Zeichnung der Samenschale auszeichnete. Es handelte sich um das Auftreten von violetten Flecken von länglicher, unregelmässiger bandförmiger Gestalt. Diese Streifen, wie man sie nennen kann, erinnern sowohl in ihrer Form wie in ihrer Verteilung auf der Samenschale ganz an die Streifung der Testa bei *Phaseolus vulgaris*, wo sie durch die Wirkung des Gens *S* in dominanter Form bedingt werden (vgl. K. TJEBBES und H. N. KOOIMAN, 1919 und 1921).

In Fig. 1 sind drei gestreifte Samen von *Pisum sativum* abgebildet. Das Bild zeigt, dass die Testa seitlich betrachtet, auf hellerem Grunde einen, zwei oder mehrere Streifen in dunklerer Farbe aufweist, die immer gewisse Unregelmässigkeiten zeigen und bald hier, bald da unterbrochen, zum Teil in kleinere Flecken aufgelöst erscheinen. Wie ferner ersichtlich verlaufen die Streifen stets ziemlich deutlich gekrümmt konzentrisch um das Hilum, von deren unmittelbarer Nähe sie ihren Ursprung nehmen. Letzteres ist an dem linken Samen in Fig. 1, der von der Hilumseite abgebildet ist, zu sehen. Die Farbe der in Frage stehenden Streifen ist eine intensiv violette, die namentlich bei nicht marmorierten Samen sehr stark zutage tritt. Bei marmorierten Samen ist sie weniger stark ausgebildet und bei nicht gut ausgereiften solchen Samen bedarf es mitunter einer genauen Untersuchung um dieselben nicht zu übersehen.

Fig. 2 zeigt drei gestreifte Samen von *Phaseolus vulgaris*. Ein Vergleich mit den Erbsensamen in Fig. 1 zeigt unmittelbar, dass die Streifung, abgesehen von der durch die Samenform bedingten Gestaltung, ganz gleichen Charakter aufweist. Vielleicht handelt es sich um homologe Gene, die bei *Pisum sativum* und *Phaseolus vulgaris* die

Testastreifung bedingen. Ganz die gleiche Streifung ist mir auch noch bei *Phaseolus multiflorus* bekannt und wahrscheinlich dürfte sie auch noch bei anderen Leguminosen-Samen anzutreffen sein. Bei *Phaseolus* kommt diese Streifung in verschiedenen Farben vor, was auf die Wirkung wechselnder Kombinationen von Testafarbgenen zurückzuführen ist.

Um die Vererbung der Testastreifung bei *Pisum* zu studieren sind

Fig. 1. Drei Samen von *Pisum sativum* mit gestreifter Testa.

Fig. 2. Drei Samen von *Phaseolus vulgaris* mit gestreifter Testa. Der linke entspricht meiner Linie 9 aus Souvenir de Deuil, die beiden rechten einer als »*Dolichos Zebra*« bezeichneten Bohnenvarietät (Linie 145).

bisher sechs Kreuzungen ausgeführt worden. Hier soll über eine derselben, Nr. 233, berichtet werden, von der 1936 die zweite Generation gebaut worden ist. Kreuzung 233 wurde ausgeführt zwischen Linie Nr. 58 aus der deutschen dickwandigen Zuckererbsensorte Graue Posthörnchen und Linie Nr. 234 mit gestreifter Samenschale. Linie 58 ist folgendermassen zu charakterisieren: Keimblätter gelb, nicht runzelig. Samenform indent, Samenschale gleichmässig gelblichbraun bis rötlichbraun marmoriert und mit zuweilen schwer zu erkennbaren violetten Punkten. Hilum hellbraun. Blüten von normalem Bau, Blütenblätter

purpurfarbig. Hülsen grün, dickwandig, konkav gekrümmt, mehr oder weniger spitz, ohne Membran, im reifen Zustand mit fleckenweise ausgebildeter sehr dünner Membran. Hoher Wuchs. Auf Grund dieser Eigenschaften und bisher mit L. 58 in Kreuzungen erhaltener Resultate ergibt sich folgende Genenformel:

A Ar B M F Oh pl R I P v n Bt Cp Le.

Linie 234 hat folgende Eigenschaften: Keimblätter gelb, nicht runzelig, Samenform etwas gepresst rundlich bis indent, Samenschale grünlichgrau, nicht marmoriert und mit stets deutlichen violetten Punkten. Hilum schwarz. Blüten von normalem Bau, Blütenblätter purpurfarbig. Hülsen grün, dünnwandig, gerade, mit stumpfem Ende und starker Membran (Kneifelerbsentypus). Hoher Wuchs. Zufolge dieser Eigenschaften ergibt sich für L. 234 folgende Genenformel:

A Ar B m F Oh Pl R I P V N Bt Cp Le.

Laut den vorstehend mitgeteilten Formeln ist in F_1 Heterozygotie in den Genpaaren *M—m*, *Pl—pl*, *V—v* und *N—n* zu erwarten. Die an F_1 beobachteten Eigenschaften standen hiermit in Übereinstimmung; die vier in Rede stehenden Gene manifestierten sich stets der dominanten Form entsprechend. In bezug auf die neue hier untersuchte Eigenschaft »gestreifte Samenschale» konnte festgestellt werden, dass sämtliche auf F_1 erhaltenen Samen diese zeigten. Die Eigenschaft Streifung der Testa scheint demnach über ungestreifte Testa klare Dominanz aufzuweisen. Erwähnt sei, dass die violette Farbe der Streifung auf den marmorierten Samen der F_1-Generation schwächer ausgebildet ist als auf den nicht marmorierten des einen Elters, L. 234. Bei weniger gut ausgereiften Samen erfordert die sichere Erkennung der Streifung, wie bereits einleitend erwähnt, eine genauere Untersuchung.

In der zweiten Generation fand eine Spaltung in Individuen mit gestreifter und solche mit nicht gestreifter Testa sowie in den oben erwähnten vier Genpaaren statt. Für die Aufspaltung hinsichtlich Streifung der Testa sind die Resultate in Tabelle 1 wiedergegeben. Die erhaltenen Spaltungszahlen zeigen eine ungewöhnlich gute Übereinstimmung mit dem monohybriden Verhältnis 3 gestreifte : 1 ungestreift. Das hier wirksame Genpaar bezeichne ich mit Hinblick auf den Samencharakter der rezessiven Form mit *Ast—ast*, abgeleitet von *astriata* = nicht gestreift.

Im Zusammenhang mit der Besprechung der Testa-Streifung verdient erwähnt zu werden, dass ich in einer Kreuzung (Nr. 25) an vier Individuen je einen Samen angetroffen habe, die einen violetten Fleck

von der Form und Lage aufwiesen, wie er am rechten Samen in Fig. 1 zu sehen ist. Ein Unterschied schien nur darin zu bestehen, dass die Begrenzung des violetten Streifens hier verwaschen war, während sie bei den gestreiften Samen der Linie 234 und der in Kreuzung 233 ausspaltenden solchen scharf begrenzt erscheinen. Ein Aufziehen von Pflanzen aus den erwähnten vier Samen hat ergeben, dass es sich höchst wahrscheinlich um einen Beginn des sogenannten *Obscuratum*-Phänomens handelte, denn die Nachkommen bildeten nur Samen ohne jegliche Streifung der Testa aus. Diese Erscheinung hatte also nichts mit der Wirkung des Gens *Ast* zu tun.

TABELLE 1. *Die Spaltung des Eigenschaftspaares gestreifte—ungestreifte Samenschale in F_2 von Kreuzung Nr. 233.*

Familien-Nr.	Individuen mit gestreifter Samenschale	Individuen mit ungestreifter Samenschale	Summe Individuen
8487	52	25	77
8488	59	16	75
8489	36	13	49
8490	51	15	66
8491	42	14	56
8492	71	19	90
8493	7	1	8
Summen:	318	103	421
D/m für 3:1 =	0,25	—	—

Das Wesen des *Obscuratum*-Phänomens besteht höchst wahrscheinlich darin, dass in der Samenschale gewisser Samen, meistens einer Hülse oder jedes zweiten Samens einer Hülse oder auch nur der einen Hälfte jedes oder jedes zweiten Samen einer Hülse, ein physiologischer Zustand (Überschreitung eines bestimmten Schwellenwertes) erreicht wird, bei dem die sonst nur in Punkten auftretende violette Farbe (abhängig von der Anwesenheit von *F*) sich über die Testa ausbreitet. Diese Ausbreitung der violetten Farbe scheint, nach meinen Beobachtungen an sowohl *Pisum* wie *Phaseolus* zu urteilen, stets in unmittelbarer Nähe des Hilums zu beginnen. Es wäre hierbei denkbar, dass die Ausbreitung in ihrem ersten Beginn in einem oder mehreren Streifen stattfindet, die hierzu in irgendeiner Weise präformiert sind und deren normale Violettfärbung durch das Gen *Ast* bedingt wird.

Über das *Obscuratum*-Phänomen bei *Pisum* und *Phaseolus* berichten u. a. B. KAJANUS (1913 a, 1913 b, 1914) und K. TJEBBES (1923).

Das Originalmaterial der genetischen Analyse in den fünf Genpaaren *V—v*, *N—n*, *M—m*, *Pl—pl* und *Ast—ast* ist in Tabelle 2 mitgeteilt. Insgesamt wurden in F_2 500 Samen ausgesät, die jedoch nur 421 in allen Eigenschaften beurteilbare Individuen entwickelt hatten,

TABELLE 2. *Die Aufspaltung der fünf Genpaare V—v, N—n, M—m, Pl—pl und Ast—ast in F_2 von Kreuzung 233.*

Familie-Nr.	*V*, mit starker Hülsenmembran																Summen
	N, dünne Hülsenwand								*n*, dicke Hülsenwand								
	M				*m*				*M*				*m*				
	Pl		*pl*		*Pl*		*pl*		*Pl*		*pl*		*Pl*		*pl*		
	Ast	*ast*	*Ast*	*ast*	*Ast*	*ast*	*Ast*	*ast*	*Ast*	*ast*	*Ast*	*ast*	*Ast*	*ast*	*Ast*	*ast*	
8487	11	4	3	3	7	6	1	3	13	1	2	—	4	2	—	1	61
8488	10	3	10	4	3	—	4	1	6	—	3	—	6	3	—	1	54
8489	12	4	1	2	8	—	1	1	1	3	—	1	1	—	1	—	36
8490	16	8	5	3	6	1	4	—	5	1	—	1	2	—	1	—	53
8491	12	7	6	1	4	1	1	—	7	1	—	1	1	1	—	3	45
8492	22	7	5	4	8	—	—	3	13	1	—	—	4	—	2	—	69
8493	1	—	2	1	—	—	—	—	—	—	—	—	1	—	—	—	5
Summen:	84	33	32	18	36	8	11	8	45	7	5	3	19	5	7	2	323
	V, mit sehr dünnen Hülsenmembranflecken																
8487	6	1	3	—	2	1	—	—	1	1	—	1	—	—	—	—	16
8488	9	2	2	1	2	1	—	—	3	—	—	—	1	—	—	—	21
8489	9	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	13
8490	8	—	—	—	2	—	—	—	1	1	1	—	—	—	—	—	13
8491	3	1	1	1	1	—	—	—	2	—	—	—	1	1	—	—	11
8492	8	2	3	1	2	1	1	—	3	—	—	—	—	—	—	—	21
8493	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	3
Summen:	45	7	10	4	10	3	1	—	10	2	1	1	2	1	—	1	98
Total:	129	40	42	22	46	11	12	8	55	9	6	4	21	6	7	3	421

was einem Prozent von 84,2 entspricht. Für die Beurteilung der Sicherheit von eventuell gefundenen schwächeren Koppelungen ist diese Zahl von Bedeutung.

Die ausser *Ast—ast* studierten vier Genpaare zeigten für sich gleichfalls klare monohybride Spaltung, indem folgende Spaltungsverhältnisse resultierten.

Gefunden: 323 *V* : 98 *v* Gefunden: 310 *N* : 111 *n*
Erwartet: 315,75 » : 105,25 » Erwartet: 315,75 » : 105,25 »
D/m für 3 : 1 = 0,82 D/m für 3 : 1 = 0,65

Gefunden: 307 *M* : 114 *m* Gefunden: 317 *Pl* : 104 *pl*
Erwartet: 315,75 » : 105,25 » Erwartet: 315,75 » : 105,25 »
D/m für 3 : 1 = 0,99 D/m für 3 : 1 = 0,14

Wie ersichtlich ist die statistische Sicherheit für sämtliche Spaltungsverhältnisse eine gute. Es folgen nun kurz zahlenmässig wiedergegeben die in Kreuzung Nr. 233 festgestellten dihybriden Spaltungsverhältnisse.

Gefunden: 230 *V N* : 93 *V n* : 80 *v N* : 18 *v n*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 0,67 + 1,76 + 0,13 — 1,67

Gefunden: 227 *V M* : 96 *V m* : 80 *v M* : 18 *v m*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 0,96 + 2,13 + 0,13 — 1,67

Gefunden: 237 *V Pl* : 86 *V pl* : 70 *v Pl* : 18 *v pl*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = + 0,01 + 0,88 — 1,12 — 1,67

Gefunden: 239 *V Ast* : 84 *V ast* : 79 *v Ast* : 19 *v ast*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = + 0,22 + 0,63 + 0,01 — 1,47

Gefunden: 233 *N M* : 77 *N m* : 74 *n M* : 37 *n m*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 0,37 — 0,24 — 0,62 + 2,15

Gefunden: 226 *N Pl* : 84 *N pl* : 91 *n Pl* : 20 *n pl*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 1,06 + 0,63 + 1,50 — 1,27

Gefunden: 229 *N Ast* : 81 *N ast* : 89 *n Ast* : 22 *n ast*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 0,77 + 0,26 + 1,25 — 0,87

Gefunden: 233 *M Pl* : 74 *M pl* : 84 *m Pl* : 30 *m pl*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 0,37 — 0,62 + 0,63 + 0,74

Gefunden: 232 *M Ast* : 75 *M ast* : 86 *m Ast* : 28 *m ast*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = — 0,47 — 0,49 + 0,88 + 0,34

Gefunden: 251 *Pl Ast* : 66 *Pl ast* : 67 *pl Ast* : 37 *pl ast*
Erwartet: 236,81 » » : 78,94 » » : 78,94 » » : 26,31 » »
D/m für
9 : 3 : 3 : 1 = + 1,39 — 1,62 — 1,49 + 2,15

Ein Studium der oben angeführten dihybriden Spaltungsverhältnisse ergibt, dass vielleicht mit Ausnahme des zuletzt angeführten keines zur Annahme einer Koppelung berechtigt. Diese letzte Spaltung in den beiden Genpaaren *Pl—pl* und *Ast—ast* zeigt das charakteristische Defizit für die zwei neuen Genkombinationen *Pl ast* und *pl Ast* und einen entsprechenden Überschuss für die beiden Elterntypen *Pl Ast* und *pl ast*. Eine Berechnung des Crossingover-Wertes ergibt 39,3 %. Die Zahlen scheinen also recht entschieden für das Vorhandensein einer Koppelung zu sprechen. Als sicher will ich eine solche indes keineswegs betrachten, da von den ausgesäten Samen, wie bereits früher erwähnt, sich nur 84,2 % zu in allen Eigenschaften beurteilbaren Pflanzen entwickelt haben. Und was im Rest, den 15,8 %, verborgen ist, darüber zu entscheiden fehlen jederlei Anhaltspunkte. Sicheren Bescheid in derartigen Fällen bekommt man gewöhnlich nur durch das Studium einer Kreuzung wo im in Frage stehenden Chromosom drei (oder mehr) Genpaare spalten.

SUMMARY.

1. The author describes a *Pisum* variety with violet striations on the seed coat. The striations in question exhibit the same character as those in *Phaseolus vulgaris* caused by the *S* gene.

2. A study of the inheritance of these striations in *Pisum* shows that they are conditioned by the dominant form of a gene, which the author indicates by the symbol *Ast* (derived from *astriata*=not striated). Thus in F_2 a monohybrid segregation of the said character has been ascertained.

3. At the same time as the segregation of the gene-pair *Ast—ast* the segregation in *V—v*, *N—n*, *M—m* and *Pl—pl* was also studied. It was possible to ascertain a weak linkage between the genes *Pl* and *Ast* (crossing-over percentage = 39,3). The author, however, does not consider that this linkage has been established for certain, for of 500 seeds in F_2 sown only 421 plants, possible of being judged in all properties, were obtained.

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CHROMOSOME NUMBERS IN PETUNIA

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CROSSINGS between *Petunia* forms having different chromosome numbers have been carried out at Hilleshög since 1930, when the late Dr. KLAAS TJEBBES procured a number of commercial varieties of *Petunia* and made crossings between diploid and tetraploid forms. In the following year the triploids obtained in these crosses were back-crossed with the parents. In 1933 Dr. TJEBBES very generously handed over his *Petunia* material to me for further cytological analysis. In addition I had the privilege to take over a number of root fixations of the material cultivated in 1933. These fixations had been performed by Dr. MÜNTZING. Along with the purely Mendelian analysis of the diploid *Petunia* material I have since then continued crossings between forms with different chromosome numbers. In the present paper I shall give, by means of a number of tables, a summarized survey of the results hitherto obtained in this field. In so doing I shall dwell as little as possible on the external morphology of the plants under discussion. I hope to have an opportunity of dealing with these matters in a future communication.

The original material consisted of the following commercial forms: pure white *nyctaginiflora*, a white form with large, more flaccid flowers, which I generally call »large flowered white», a *violacea* type, a »Rathaus» and a form with white petals having blue veins. All these were diploids with $2n = 14$. Further, there was a purple giant form, »*superbissima*», and another white-flowered giant form, »Soleil d'or», with $2n = 28$.

The chromosome numbers in the specimens described below were determined in root-tips fixed in Navashin's fluid. Gentian violet was used exclusively as the stain. The determinations of the chromosome numbers in 1930—31 were made by Dr. MÜNTZING and some of the results have already been published (TJEBBES, 1932).

I. DIPLOIDS AND TETRAPLOIDS.

Selfings in diploids, like crosses between diploids, have always yielded a progeny with the same chromosome number as the parents.

That is not the case, however, in self-fertilisation and inter-crossing of tetraploid forms. In 1931 in particular Dr. TJEBBES carried out numerous self-fertilisations of tetraploids. A variation in the chromosome number in the progeny was to be expected owing to the quadrivalent formation observed at meiosis and the subsequent unequal chromosome distribution and elimination. Since then several such progenies have

TABLE 1. *Progenies of Tetraploids.*

Year	No.	Mother plant		Pollen plant		Progeny 2n =				
		Form	2n	Form	2n	27	28	29	30	Total
1931	182	red, *superbissima*	28	selfed	28	1	7	1	—	9
»	183	»	»	»	»	—	2	—	—	2
»	184	»	»	»	»	—	1	—	—	1
»	185	»	»	»	»	—	2	1	—	3
»	186	»	»	»	»	—	3	—	—	3
»	188	»	»	»	»	—	—	—	1	1
»	190	»	»	»	»	—	3	—	—	3
»	192	»	»	»	»	—	1	—	—	1
»	194	white, Soleil d'or	»	»	»	2	2	—	—	4
»	195	»	»	»	»	—	2	—	—	2
»	196	»	»	»	»	—	3	—	—	3
»	201	»	»	»	»	—	2	—	—	2
»	202	red, *superbissima*	»	white, Soleil d'or	»	—	2	—	—	2
»	203	»	»	»	»	—	2	1	—	3
»	204	»	»	»	»	1	2	—	—	3
»	205	white, Soleil d'or	»	red, *superbissima*	»	1	1	—	—	2
1932	537	red	27	selfed	27	5	5	—	—	10
»	538	»	29	»	29	—	1	—	—	1
»	540	white	27	white	27	1	2	—	—	3
»	541	red, with white spots	27	selfed	»	5	4	—	—	9
1934	247	red	28	»	28	7	15	3	—	25
1936	678	»	30	»	30	—	2	2	—	4
					Total:	23	64	8	1	96

M = 27,9

been investigated. Table 1 gives a summary of the results. The variation in the somatic chromosome numbers present was between 27 and 30. The 28-class was the most numerously represented, the next being the 27-class. In this region of the chromosome area the aneuploid variants are not appreciably less vital than the euploid forms. They cannot be morphologically distinguished from the latter.

Crosses between tetraploids and diploids were attempted on a rather large scale. In the direction 4x × 2x these crosses succeeded

much more easily than in the reciprocal direction. Still, sterility is high also here. From Table 2 it will be seen that only 26 plants were obtained from 11 crossed flowers in the direction 4x × 2x. In complete fertility as many as 200 plants can be raised after a single crossed flower. As was to be expected, most of the plants obtained in these crosses were triploids or 3x ± 1 plants. Some individuals with a divergent chromosome number occurred however. In one cross there arose a plant with 18 chromosomes. Like other *Petunia* plants with this chromosome number, this plant was exceedingly frail and had small flowers. Never-

TABLE 2. *Tetraploid* ♀ × *Diploid* ♂.

Year	No.	Mother plant		Pollen plant		Progeny 2n =						
		Form	2n	Form	2n	18	20	21	22	29	31	Total
1931	151	red, *superbissima*	28	*violacea*	14	—	2	5	—	—	—	7
»	158	»	»	*nyctaginiflora*	»	—	1	5	—	—	—	6
»	159	»	»	»	»	1	—	—	—	—	—	1
»	174	»	»	large-flowered, white	»	—	—	1	—	—	1	2
»	179	»	»	Rathaus	»	—	—	—	—	1	—	1
»	217	»	»	*violacea*	»	—	—	1	—	—	—	1
1936	650	white, red veined	»	*nyctaginiflora*	»	—	—	3	—	—	—	3
»	660	red, white spotted	»	»	»	—	—	1	1	—	—	2
»	663	red	»	»	»	—	1	—	—	—	—	1
»	670	purple	»	»	»	—	—	1	—	—	—	1
»	680	red	»	»	»	—	—	1	—	—	—	1
					Total:	1	4	18	1	1	1	26

M = 21,0

theless it produced progenies when crossed (cf. Table 5). Further, 2 quasi-tetraploids were obtained, the results of unreduced male gametes. Such plants were also obtained by MATSUDA (1935) in this cross. A particularly large number of tetraploids were obtained, however, by KOSTOFF and KENDALL (1931), 16 out of 20 plants examined being pure tetraploids. On the other hand, STEERE (1932) obtained exclusively triploids in this cross.

The reciprocal cross, 2x × 4x, yielded quite different results. As known, STEERE (l. c.) made some interesting observations in this cross. In the first place the cross succeeded very easily, quite a large number of plants being obtained (1348 from 15 capsules), in the second place about half of the plants were exactly like the diploid mother plant, while the remainder was more or less intermediary between the parents.

Randomly selected and fixed plants all proved to be diploids, whence all the offspring was considered to be diploid. With regard to the origin of these plants the author writes (p. 341): »They cannot have arisen parthenogenetically, but must have contained a certain number of chromosomes contributed by the pollen parent, as the resemblance

TABLE 3. *Diploid* ♀ × *Tetraploid* ♂.

Year	No.	Mother plant		Pollen plant		Progeny 2n =						
		Form	2n	Form	2n	14	21	27	28	29	30	Total
1931	162	*violacea*	14	white, Soleil d'or	28	5	—	—	—	—	—	5
1934	250	*nyctaginiflora*.....	»	purple	25	—	1	—	—	—	—	1
»	270	»	»	»	30	—	1	—	—	—	—	1
»	271	»	»	»	30	—	—	—	—	—	1	1
»	276	»	»	»	28	1	—	—	—	—	—	1
1936	686	large-flowered, white.....	»	red.....	28	—	—	1	3	1	—	5
»	688	»	»	»	30	—	—	—	2	—	—	2
					Total:	6	2	1	5	1	1	15

M = 22,1

TABLE 4. *Progenies in 1932 from the cross 2x × 4x.*

Mother plant		Pollen plant		Progeny		
Form	2n	Form	2n	No.	Number of plants	Type of plants
violacea	14	red, *superbissima*	28	517	3	pure *violacea*
»	»	»	»	520	4	»
nyctaginiflora.....	»	»	»	522	1	pure *nyctaginiflora*
»	»	»	»	523	1	purple
»	»	»	»	524	4	3 *nyct.*, 1 purple
F_1 *viol.* × *nyct.* ...	»	»	»	530	2	2 purple
large-flowered, white.....	»	»	»	534	4	3 white, 1 purple

in habit and flower color showed. Different proportions of each set of parental chromosomes were apparently present in these F_1 plants, since there was such a wide range of variation . . .». It thus appears that an entirely new mechanism either in the meiosis of the pollen plant or in the fertilisation must have come into use, with the result that only 7 of the 14 chromosomes present in the pollen of the tetraploids had functioned.

MATSUDA, too, (l. c.) obtained only diploids in such a cross (85 plants). All these plants, however, agreed entirely with the mother plant and may consequently have been produced parthenogenetically. DERMEN (1931), on the other hand, obtained 3 normal triploids in this cross.

Crossings of this kind were made by Dr. TJEBBES already in 1930. Only in one of them were the chromosome numbers of the progeny known (v. Table 3). All 5 progeny plants were diploids and like the mother plant. These crossings were repeated in the following years, the numbers given in Table 4 being progenies from the crosses made in 1931. From this table it will be seen that in certain cases (in Nos. 523, 524 and 534) the characters of the father plant are apparent in the offspring. As the white-flowered mother plants were inbred for 2 years and could consequently be regarded as homozygous for flower colour, the variations in colour in the progeny must be considered due to the influence of the father plants. Unfortunately the chromosome numbers of these progenies are not known and in only one case has a new generation been cultivated from them. Fortunately, however, this was an important number, viz. 534. Of this number the progenies of all 4 plants were cultivated after self-fertilisation in 1933. All the 3 white plants yielded uniform white progenies, the offspring of the purple-coloured plant, No. 34, segregated into 8 coloured and 3 white plants. 7 of these have been examined cytologically and were found to have the following chromosome numbers:

2n:	14	14 + fr.	15
Number of plants:	5	1	1

From this we venture to conclude that the coloured mother plant was not a pure diploid, but probably had a higher chromosome number, presumably near the triploid number.

The cross was again made by me in 1933, this time the experiments were carried out on a somewhat larger scale. As diploids were used only *nyctaginiflora* plants, which had been selfed for several generations, and were known to give only white progenies. During these crossings the plants were kept in closed isolation cells. 58 flowers were castrated and pollinated with pollen from red *superbissima* plants with the chromosome numbers 2n = 27—31. Only 24 flowers showed any evidence of seed-setting. The mature seeds were thin and transparent. Nevertheless, all seeds obtained were put to germination even if they appeared to be empty. Only 3 seeds germinated, 2 of which were from the same

cross but from different seed-capsules. In this case the father plant had 30 chromosomes. The two plants thus obtained, Nos. 270 and 271, flowered in 1934. They mostly resembled the father plant, were large and tall and had red flowers. Their chromosome numbers were 21 and 30 respectively. The third seed in this cross gave rise to a white diploid plant similar to the mother plant. Another crossing of the same kind made in the same year between a white diploid and a hypotetraploid ($2n = 25$) gave a red triploid plant.

This kind of cross was again repeated to about the same extent in 1934. This time, however, another white diploid, viz. the large-flowered white, was crossed with red tetraploids. Seeds were obtained in 2 capsules, which were not sown until the winter of 1935, when Nos. 686 and 688, consisting of 5 and 2 plants respectively, were obtained. All these plants were tetraploids or $4x \pm 1$. They were all coloured and were characterized by the flower colour not being evenly distributed, there being a larger or smaller number of white spots. As has been ascertained in numerous crosses between different diploids, the large-flowered white race has no factors for flower colour constancy, which are possessed, for instance, by *nyctaginiflora*, and that is undoubtedly the cause of the dissimilarity to the 1934 progeny in corresponding crosses.

To sum up the results of these $2x \times 4x$ crosses it should be pointed out that the singular results obtained by STEERE have not been confirmed in our material. In the first place the cross proved to be extremely difficult to carry out, perhaps a seed-setting of $1\,{}^{0}/_{000}$, and, further, among 34 plants there was not a single certain diploid with characters from both parents. On the other hand, triploids and tetraploids were formed, which in later analysis proved themselves to have arisen by fusion of a 1x egg-cell and a 2x pollen-cell or of a 2x egg-cell and a 2x pollen-cell. Whether the diploids obtained, which were exactly like the mother plant, arose by pseudogamy or whether there is some experimental error present is not easy to determine.

II. CROSSES WITH TRIPLOIDS.

Triploids have been self-fertilized and crossed reciprocally with both diploids and tetraploids. The results are summarized in Tables 5 and 6.

Self-fertilisation of triploids succeeds very seldom in *Petunia*. I therefore possess data from only one progeny of this type, consisting

altogether of 10 plants. The somatic numbers of these plants were all *below* the triploid number and not a single triploid was reconstituted. In this case MATSUDA (l. c.) obtained a different result: of 36 plants all except one, which was presumably a diploid, had the chromosome number 26—29, the number 27 being represented by 23 plants. DERMEN (l. c.) obtained a similar result, his triploid offspring having 26—27 chromosomes (Table 9).

TABLE 5. *Crosses with Triploids.*

Year	No.	Mother plant		Pollen plant		Progeny 2n =							
		Form	2n	Form	2n	14	15	16	17	18	19	20	Total
1932	450	violet, veined.....	20	violet.....	21	3	3	2	1	1	—	—	10
1932	454	violet.....	21	*violacea*	14	1	1	2	1	1	—	—	6
»	458	violet.....	»	*nyctaginiflora*...	»	—	—	1	—	—	—	—	1
»	459	pale violet	»	blue, veined ...	»	1	2	1	—	—	—	—	4
»	463	low, violet, veined	18	*violacea*	»	4	5	1	—	—	—	—	10
»	464	dark purple	21	*nyctaginiflora*...	»	2	2	1	—	—	—	—	5
»	473	purple violet.....	»	F_1 *viol.* × *nyct.*	»	1	3	1	1	—	—	—	7
»	476	veined	»	blue, veined ...	»	1	3	—	—	1	—	—	5
1934	242	white	»	purple	»	2	2	3	—	1	—	—	8
»	244	»	»	Rathaus	»	—	6	5	1	—	—	—	12
»	246	»	»	*nyctaginiflora*...	»	2	4	1	—	1	1	—	9
1936	668	red.....	»	»	»	—	—	—	—	—	—	1	1
»	671	purple	»	»	»	2	1	2	1	—	—	—	6
»	675	red.....	»	»	»	1	1	—	—	—	—	—	2
»	676	»	»	»	»	1	2	3	—	—	—	—	6
»	677	»	»	blue veined ...	»	—	3	3	1	1	—	—	8
					Total:	18	35	24	5	5	2	1	90
1934	204	*nyctaginiflora* ...	14	white	21	10	—	—	—	—	—	—	10
»	251	»	»	»	»	26	—	—	—	—	—	—	26
1936	681	blue, veined	»	red.....	»	5	—	—	—	—	—	—	5
					Total:	41	—	—	—	—	—	—	41

M = 15,4

M = 15,5

M = 14,0

The cross 3x × 2x (Table 5) gave all numbers between 14 and 20. The class most numerously represented was the trisomic. An interesting feature is the presence of a large number of 16-chromosome plants, which were more numerous than diploids. Moreover, 10 plants had 17—18 chromosomes and 3 plants had 19—20 chromosomes. In this cross MATSUDA obtained 3 plants with 19, 20 and 21 chromosomes respectively. The difference here, as compared with my results, is some-

what the same as in the self-fertilisation of triploids, where in my material chiefly gametes with 7—9 chromosomes had functioned, whereas in MATSUDA's material the functioning gametes had 12 to 15 chromosomes.

The reciprocal cross, diploid × triploid, gave a strikingly different result, the 41 plants occurring in 3 different numbers being without exception diploids. There was not a single plant with supernumerary chromosomes. Here we have a new example of a phenomenon known from several earlier investigations (e. g. *Oenothera*, VAN OVEREEM, 1921; *Zea*, MC CLINTOCK, 1929; *Allium*, LEVAN, 1936), viz. that if a triploid plant is fertilized with pollen from a diploid several, frequently all, chromosome classes between 2x and 3x will be developed in the progeny,

TABLE 6. *Triploids and Tetraploids.*

Year	No.	Mother plant		Pollen plant		Progeny 2n =							
		Form	2n	Form	2n	24	25	26	27	28	31	Total	
1932	481	violet	21	purple	28	1	1	—	2	—	—	4	
»	497	»	»	»	»	—	1	—	1	2	—	4	
»	500	purple	»	»	29	—	—	1	2	4	1	8	
1934	245	white	»	red, white spotted	28	—	-	—	—	1	—	1	
				Total:		1	2	1	5	7	1	17	M = 27,2
1932	485	red	28	pale violet	21	—	—	—	—	4	—	4	M = 28,0

on the other hand if a diploid plant is fertilized with pollen from a triploid *no* plants are produced with 2x + 1, 2, 3 and so on, but only 2x plants and occasionally also 3x, 3x—1, 2 and 3 (as was the case in *Allium*). DERMEN's results from this cross run along the same lines, although not so extreme as mine. Amongst 51 plants he obtained 39 diploids and 12 trisomes.

In crosses between triploids and tetraploids there are only a few progeny plants (v. Table 6), 17 in one direction and 4 in the other. They approach the tetraploid number (24—31). In a cross 3x × 4x there occurred the highest chromosome number found so far in our *Petunia* material, viz. 2n = 31. In the same cross DERMEN once obtained a pentaploid plant (2n = 35). In the direction 4x × 3x I obtained only pure tetraploids. In this cross MATSUDA found a greater dispersion of the chromosome numbers (24—29). 17 of the 31 plants examined by him were tetraploids. It is obvious, however, that in these

crosses between triploids and tetraploids the triploids and the classes immediately above the triploid number are less readily realizable combinations than the classes round the tetraploid number.

III. TRISOMES AND DIPLOIDS.

In progenies from triploids there were found, as mentioned, a great many trisomes, such as were trisomic for whole chromosomes as well as

TABLE 7. *Crosses with Trisomes.*

Year	No.	Mother plant		Pollen plant		Progeny 2n=			
		Form	2n	Form	2n	14	15	Total	
1934	210	pale purple	15	free-flowering	—	2	2	4	[1]
»	225	violet	»	»	—	1	1	2	[1]
»	228	white, veined	»	»	—	11	2	13	[1]
»	230	»	»	red	15	10	3	13	[1]
»	296	»	»	selfed	»	3	2	5	[1]
1936	652	white	»	»	»	4	3	7	[1]
				Total:		31	13	44	M = 14,3
1934	226	violet	15	*nyctaginiflora*	14	9	—	9	[1]
»	231	white, veined	»	»	»	11	7	18	[1]
»	289	violet	»	large-flowered white	»	8	2	10	
				Total:		28	9	37	M = 14,2
1934	201	*nyctaginiflora*	14	white, veined	15	16	—	16	[1]
»	202	»	»	violet	»	7	—	7	
»	203	»	»	»	»	10	—	10	[1]
»	205	»	»	blue violet	»	6	1	7	[1]
»	212	pale violet	»	violet	»	16	—	16	
»	280	*nyctaginiflora*	»	white, veined	»	15	—	15	[1]
»	319	white	»	red violet	»	10	—	10	[1]
				Total:		80	1	81	M = 14,0

such as had only one diminutive chromosome extra. The trisomes exhibit good vitality but are considerably more sterile than the diploids. Certain of the trisomic classes can be morphologically identified, and in a future work I shall give a description of them.

The chromosome numbers of sundry trisomic progenies are collected in Table 7. In several of them there also occurred fragmented chromosomes, but these fragments are not taken into account in the

[1] In these numbers there also occurred diminutive chromosomes.

table. A report of the appearance and behaviour of the fragments is in the press. Progenies of trisomes after selfing and free-flowering, as well as after crossing different trisomes, all exhibit corresponding chromosome numbers, i. e. they contain diploids and trisomes, the latter in smaller number. The same conditions prevail in the cross trisome × diploid. The reciprocal cross, however, gave a different result: amongst 81 plants there appeared only one trisome, all the others were diploids. Hence we seem to have here a parallel to the condition found in the diploid—triploid crosses. The egg-cells readily carry an extra chromosome, but in the pollen the 7-chromosome cells function almost exclusively, it is only exceedingly seldom that an 8-chromosome pollen-grain contributes in the origin of an embryo.

IV. THE PETUNIA POPULATION IN 1933.

It will be seen that no chromosome numbers are recorded in the preceding tables for 1933. The cause of this is that no crosses were performed in the material in 1932, only a few isolations being carried out. When I undertook the cytological analysis of the material in 1933 there were therefore only seeds after selfing and after free-flowering in the 1932 material. From Table 8 it will be seen that the 1932 material was made up of progenies after the crosses triploid × diploid (Nos. 15—25), triploid × tetraploid (Nos. 24—28 and 38—40), diploid × tetraploid (Nos. 31—34) and self-fertilized tetraploids (Nos. 29, 30 and 37). From what was demonstrated above concerning the frequency of the chromosome numbers in such progenies it can be assumed that the 1932 material contained most of the chromosome numbers between 2x and 4x + 2.

It may be of a certain interest to see how a *Petunia* population of this kind changes when it is not experimentally led in one direction or other but is allowed to take care of itself, that is, to be self-fertilized. For self-fertilisation certainly takes place frequently even in free-flowering, as the stamens in *Petunia* dehisce already in the bud-stage and early pollination has always proved to give the best results. I therefore selected for chromosome investigation 116 plants in the 1933 *Petunia* population, selecting of course chiefly such plants as, judging from their external aspect, might be suspected of exhibiting variations in the chromosome number. In Table 8, where the results are recorded, the percentage of aneuploids can therefore be considered higher than in the material in its entirety.

In what way do the results show that the chromosome numbers in the population have been changed after a year's freedom from directed crossings? The most conspicuous feature is that a good many classes of chromosome numbers have disappeared altogether, viz. the classes 17—26. There remain only diploids and two numbers above diploids, tetraploids, one number below and 2 numbers above tetraploids. Where-

TABLE 8. *Chromosome numbers in 1933.*

No.	Mother plant	Selfing or free-flowering	2n numbers of progeny							Total
			14	15	16	17—26	27	28	29	30	
15	triploid × diploid.....	free	3	3	—	—	—	—	—	—	6
17	»	self	8	1	—	—	—	—	—	—	9
18	»	»	15	—	—	—	—	—	—	—	15
19	hypotriploid × diploid	»	2	3	—	—	—	—	—	—	5
20	»	»	4	3	—	—	—	—	—	—	7
21	»	»	2	2	—	—	—	—	—	—	4
22	»	»	1	3	—	—	—	—	—	—	4
23	triploid × diploid.....	free	5	—	—	—	—	—	—	—	5
24	triploid × tetraploid ...	self	—	—	—	—	—	2	1	—	3
25	tetraploid × triploid ...	»	—	—	—	—	1	5	—	—	6
26	triploid × tetraploid ...	free	—	—	—	—	—	9	—	—	9
27	»	»	—	—	—	—	—	4	—	—	4
28	»	self	—	—	—	—	1	2	—	—	3
29	tetraploid.....	»	—	—	—	—	—	2	2	1	5
30	»	»	—	—	—	—	—	4	—	—	4
31	diploid × tetraploid.....	»	5	—	—	—	—	—	—	—	5
32	»	»	—	1	—	—	—	—	—	—	1
33	»	»	5	—	—	—	—	—	—	—	5
34	»	»	5	1	1	—	—	—	—	—	7
37	tetraploid.....	»	—	—	—	—	—	5	—	—	5
38	tetraploid × triploid ...	»	—	—	—	—	—	1	—	—	1
39	»	»	—	—	—	—	—	—	1	—	1
40	»	»	—	—	—	—	—	1	—	1	2
		Total:	55	17	1	0	2	35	4	2	116

as in the preceding generation 15 different chromosome numbers may have been represented in the material there are now only 7 different chromosome numbers present. A result along these lines was of course to be expected, but it could hardly be expected that the result would be obtained so quickly.

This is of a certain importance for the understanding of natural selection in amphimictic populations with a variation in the chromosome

numbers. If, as so often happens, diploid and tetraploid forms of a species occur in nature and a crossing between these two should come about, possible back-crossings between the triploid so formed and the parents would give rise to a population containing many different chromosome numbers. This would, however, have a very ephemeral effect

TABLE 9. *Summary of chromosome numbers hitherto found within Petunia progenies.*

♀	♂	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	35	Tot.	Author
2x	2x + 1	80	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	81	LEVAN
2x + 1	2x	28	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	37	»
2x + 1	2x + 1	31	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	44	»
2x	3x	39	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	51	DERMEN
»	»	41	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	41	LEVAN
3x	2x	—	—	—	—	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	3	MATSUDA
»	»	18	35	24	5	5	2	1	—	—	—	—	—	—	—	—	—	—	—	—	90	LEVAN
3x	3x	—	—	—	—	—	—	—	—	—	—	—	—	1	3	—	—	—	—	—	4	DERMEN
»	»	1?	—	—	—	—	—	—	—	—	—	—	—	3	23	5	4	—	—	—	36	MATSUDA
»	»	3	3	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	LEVAN
3x	4x	—	—	—	—	—	—	—	—	—	—	—	—	—	11	2	—	—	—	1	14	DERMEN
»	»	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20	1	—	—	—	21	MATSUDA
»	»	—	—	—	—	—	—	—	—	—	—	1	2	1	5	7	—	—	1	—	17	LEVAN
4x	3x	—	—	—	—	—	—	—	—	—	—	1	—	1	6	17	6	—	—	—	31	MATSUDA
»	»	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	4	LEVAN
2x	4x	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	3	DERMEN
»	»	1348	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1348	STEERE
»	»	85	—	—	—	—	—	—	—	—	—	—	—	—	1	3	—	—	—	—	89	MATSUDA
»	»	6	—	—	—	—	—	—	2	—	—	—	—	—	1	5	1	1	—	—	16	LEVAN
4x	2x	—	—	—	—	—	—	1	1	—	—	—	—	—	2	16	—	—	—	—	20	KOST.etKEND.
»	»	—	—	—	—	—	—	—	25	—	—	—	—	—	—	—	—	—	—	—	25	STEERE
»	»	3	—	—	·	—	—	1	23	1	1	—	—	—	—	1	—	—	—	—	30	MATSUDA
»	»	—	—	—	—	1	—	4	18	1	—	—	—	—	—	—	1	—	1	—	26	LEVAN
4x	4x	—	—	—	—	—	—	—	—	—	—	—	1	—	—	29	1	—	—	—	31	MATSUDA
»	»	—	—	—	—	—	—	—	—	—	—	—	—	—	23	64	8	1	—	—	96	LEVAN
Total:		1683	73	26	6	7	3	8	73	2	1	2	3	6	75	173	22	2	2	1	2168	

on the composition of the population, for already in the following generation, that is, in the second generation after the triploid, practically all aneuploid forms will be eliminated, at any rate all numbers in the proximity of the triploid number. And before long the population will have returned to its original state.

The phenomenon just described has been pointed out several times

before. NISHIYAMA (1934) has experimentally demonstrated similar conditions in *Avena* crosses. In the absence of directed crosses, plants with lower numbers than the triploid are drawn down towards the diploid number (a »diminishing group») and plants with higher chromosome numbers than the triploid are drawn up towards the tetraploid number (an »increasing group»). Balance is not acquired in nature until all aneuploids have been eliminated and only diploids and tetraploids remain.

SUMMARY.

1. Crosses between tetraploid ♀ and diploid ♂ give mainly triploids, occasionally, however, unreduced pollen grains function thus giving rise to quasi-tetraploids. Table 2.

Crosses between diploid ♀ and tetraploid ♂ give diploids similar to the mother plants (pseudogamy?), triploids and tetraploids (depending on whether the pollen of the tetraploid meets a reduced or an unreduced egg-cell). Table 3.

2. Self-fertilisation of a triploid gives the chromosome numbers 14—18. Triploid ♀ × diploid ♂ gives the chromosome numbers 14—20 (14—16 being the most numerous). On the other hand, diploid ♀ × triploid ♂ gives exclusively diploid plants (selection of ♂ gametes or of zygotes). Table 5.

Triploid ♀ × tetraploid ♂ and the reciprocal cross give numbers in the proximity of the tetraploid number (24—31). Table 6.

3. In self-fertilisation, free-flowering and inter-crossing and when crossed with diploids, trisomes give diploids and trisomes in the progeny. The cross diploid ♀ × trisome ♂ gives practically diploids only (gamete or zygote selection). Table 7.

4. In the progeny after self-fertilisation and free-flowering of a population containing most of the chromosome numbers between 14 and 30 only the chromosome numbers 14—16 and 27—30 are found. By gamete or zygote selection all numbers between 17 and 26 have disappeared from the population. Table 8.

5. A summary table is given, showing the chromosome numbers hitherto found within *Petunia* both by earlier workers and in the present investigation. Table 9.

Hilleshög, October 1936.

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THE EFFECTS OF CHROMOSOMAL VARIATION IN DACTYLIS

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I. INTRODUCTION.

THE present work was started five years ago as a link in a series of investigations concerning the possibilities of experimental polyploidy in the material of cultivated plants grown at the Svalöf Plant Breeding Institute.

The genus *Dactylis* comprises only two species, *D. glomerata* L. and *D. Aschersoniana* GRÆBN. These species differ in chromosome number, *D. glomerata* being tetraploid ($2n = 28$) and *Aschersoniana* diploid ($2n = 14$). The chromosome number of the latter species was first determined by LEVAN (1930) and KATTERMANN (1931). The chromosome number of *D. glomerata* was known earlier and has been reported by several workers to be 28 (cf. MÜNTZING, 1933 a). — As reported in a previous preliminary paper (MÜNTZING, 1933 a) *Dactylis glomerata* is not cytologically stable. Quadrivalents are frequent at meiosis and individuals with deviating chromosome numbers are formed. In the progeny from a trisomic (or more correctly, pentasomic) plant with 29 chromosomes, the chromosome numbers ranged from 27 to 31. In this progeny individuals having $2n = 28$ were on an average more vigorous than plants with deviating chromosome numbers.

Since these results were published many new data have accumulated. Chromosome variation within *D. glomerata* has been further studied and progenies have been raised from *glomerata* × *Aschersoniana* hybrids. Starting from the pure species and their hybrids a rather wide chromosomal variation has been obtained, thirty different chromosome numbers being represented. In the main this material has been studied from two points of view. Firstly, in order to illucidate the effects of polyploid and aneuploid chromosome numbers, secondly in order to investigate as far as possible the real nature of the species difference *glomerata—Aschersoniana*. Since *D. glomerata* must be considered to be an autotetraploid, the second task is closely connected with the general problem concerning the evolutionary importance of autopolyploidy. This question has recently been treated in a general

way (MÜNTZING, 1936 a). The present *Dactylis* work may be regarded as a detailed analysis of a special case, which, however, is of importance for more general conclusions.

The effects of different chromosome numbers have been studied with reference to a) vigour, b) fertility (chiefly pollen fertility) and c) morphology. The latter chapter leads on to a discussion of the species differences. First of all, however, it is necessary to give an account of meiosis and variation in chromosome number.

II. MEIOSIS AND CHROMOSOME VARIATION.

1. MEIOSIS IN DACTYLIS ASCHERSONIANA.

A. REGULAR MEIOSIS.

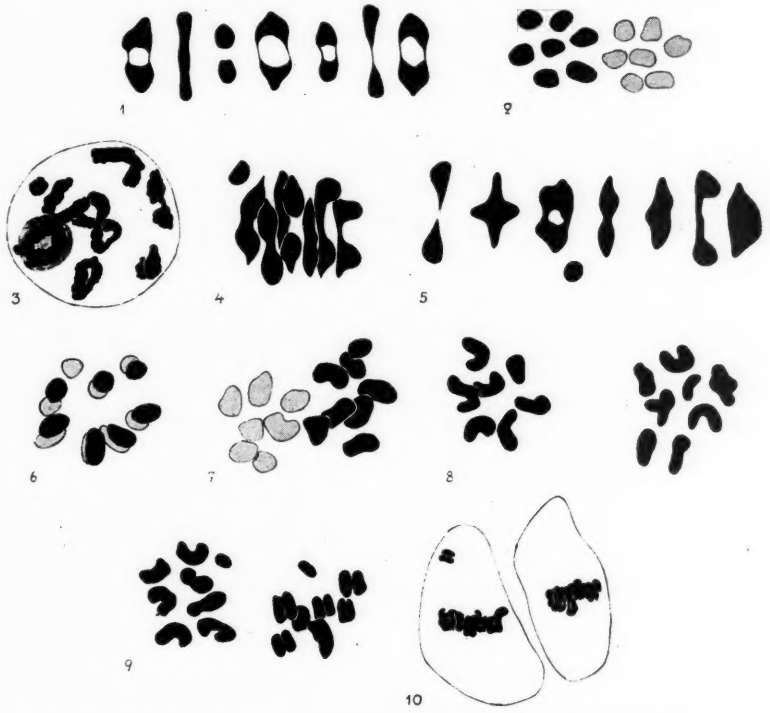
Meiosis has been studied in three plants of *D. Aschersoniana*, representing three different biotypes. One of these was selected for investigation on account of its partial pollen sterility, the other two plants had rather good pollen. In one of the fertile plants meiosis was found to be perfectly regular, judging from first metaphases with seven bivalents and first anaphases with regular disjunction, 7—7 (Figs. 1—2). In order to determine the chiasma frequency (cf. below) 73 I—M groups were examined and of these 71 were found to contain seven bivalents. In two cases, however, there were six bivalents and two univalents. The majority of fertile biotypes of *Aschersoniana* have probably the same regular meiosis.

B. TYPE WITH A FRAGMENT.

The second biotype tested was found to contain a large fragment in addition to the fourteen normal chromosomes.

At meiosis the fragment appeared to be almost as large as a normal chromosome (Figs. 3—10). Though no measurements were undertaken it is clear, however, from observations alone that the body in question represents a fragment and not a whole chromosome. This conclusion is further supported by the fact that the fragment was always free and never associated with the other chromosomes. In 35 complete diaphase nuclei there were always 7 bivalents + 1 fragment (Fig. 3) and with the exception of one group having the formula $6_{II} + 2_{I} + 1$ f the same situation was again met with in 50 I—M groups (Figs. 4—6). At first anaphase the fragment as a rule passes without division to one of the poles (Fig. 7) and is generally included in the interphase nuclei. Of 108 p. m. c., observed at this stage, 95 were quite regular, 12 contained one micronucleus in addition to the interphase nuclei and in one case there were two micronuclei. In the latter case the fragment had probably divided. That such division at I—A may occur is evident from II—M observations. Of 35 p. m. c. at this stage, in which the chromosomes of both plates could be counted, the majority

(27 cases) represented the distribution 7—8 (Fig. 8). In five cases the fragment was eliminated and outside the II—M groups, both of which consequently contained 7 chromosomes (cf. Fig. 10). In three cases, however, division had evidently occurred, each plate containing seven ordinary chromosomes + half a fragment (Fig. 9). The chromosome numbers in 108 single II—M plates were also counted. Of these 61



Figs. 1—10. Meiosis in *Dactylis Aschersoniana*. Figs. 1—2, normal *Aschersoniana* with regular meiosis. Fig. 1, I—M (7_{II}, separately drawn); Fig. 2, I—A (7—7 distribution). — Figs. 3—10, type with fragment. Fig. 3, diakinesis ($7_{II} + 1$ f); Figs. 4—5, I—M in side view ($7_{II} + 1$ f; Fig. 4, natural position, Fig. 5, separately drawn); Fig. 6, I—M, polar view ($7_{II} + 1$ f); Fig. 7, I—A (distribution 7—7 + 1 f); Fig. 8, II—M, polar view, undivided fragment in one of the metaphase groups; Fig. 9, II—M, divided fragment in each group; Fig. 10, II—M, side view, fragment eliminated. — Magnification: Figs. 1—9, 3375 ×, Fig. 10, 1688 ×.

contained seven and 47 eight bodies. — In agreement with the observations mentioned above there were seldom any lagging chromosomes at II—A. At this stage the fragment in most cases evidently divides at the same time as the other chromosomes. In consequence of this rather regular meiosis the fragment will be transmitted to most of the (male) gametes.

C. TYPE WITH ASSOCIATION OF FOUR CHROMOSOMES.

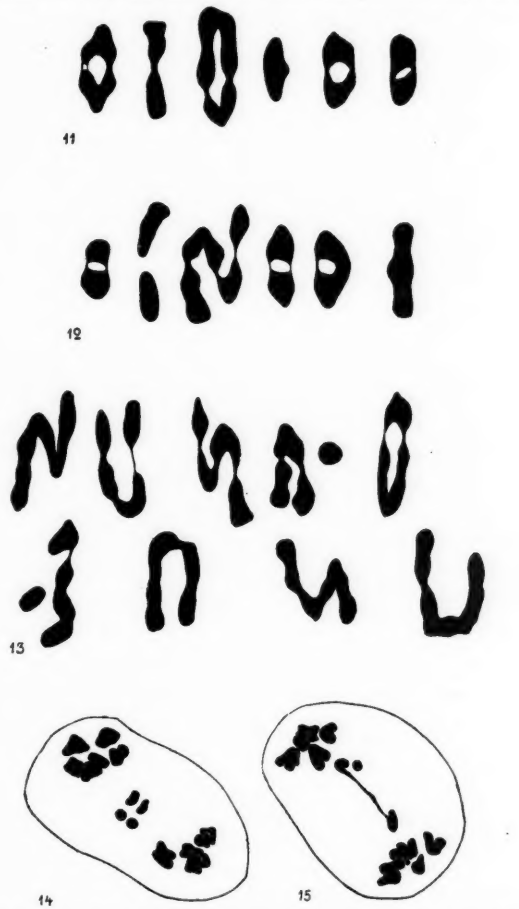
The third *Aschersoniana* type examined is characterized by partial pollen sterility and the presence of associations of four chromosomes at meiosis (Figs. 11—13). Such associations, however, do not occur regularly in each I—M group. On the contrary the presence of seven bivalents was the most frequent configuration. In a few cases the »quadrivalent» was replaced by a trivalent + a univalent and in other cells one or more of the bivalents showed non-conjunction. In a total of 182 I—M groups from two slides the following configurations were found:

Configuration	Slide No. 1	Slide No. 2	Total
7_{II}	53	47	100
$1_{IV} + 5_{II}$	42	27	69
$1_{III} + 5_{II} + 1_{I}$	2	3	5
$6_{II} + 2_{I}$	3	4	7
$4_{II} + 6_{I}$	—	1	1

Two complete I—M groups are represented in Figs. 11—12 and a number of separate associations of four chromosomes (or three + a univalent) in Fig. 13. As a rule the »quadrivalents» are represented by open chains (straight or in zig-zag arrangement as in Fig. 12) and seldom as closed rings. Such rings (cf. Fig. 11) do occur however. Of 63 quadrivalents observed (in side view) 55 were chains and 8 rings. Thus in this *Aschersoniana* type about 41 per cent of the I—M groups contain an association of four (or three) chromosomes, and of these associations about 13 per cent occur as closed rings, 87 per cent as chain configurations. These facts demonstrate that the plant in question must be heterozygous for a structural change and, as rings are formed, this change must be a reciprocal translocation. In analogy to the results obtained by BURNHAM (1932) and CLARKE and ANDERSON (1935) it may further be concluded that in this case the interchange must involve rather small segments varying somewhat in size. I have observed other similar cases in the genus *Galeopsis* (unpublished data). From this and other evidence it seems probable that such slight structural changes are of rather frequent occurrence, though the cytological symptoms are easily overlooked.

Before leaving this *Aschersoniana* type it should be mentioned that, as a rule, disjunction was found to be regular at I—A. Of 18 p. m. c., in which the chromosomes could be counted, the distribution 7—7 was found in 15 cases. In one case, however, there was division of one univalent and in two cases of two univalents (Fig. 14), the distributions in these cases being 7—1—6 and 6—2—6. Of 62 additional

cells, in which the chromosome number could not be counted, 3 showed division of one univalent and 3 of two univalents. Another exceptional case, the simultaneous



Figs. 11—15. Meiosis in *Dactylis Aschersoniana* (continued). Type with association of four chromosomes. Figs. 11—12, complete first metaphase groups in side view (separately drawn), $1_{IV} + 5_{II}$; Fig. 13, associations of four chromosomes (or three chrs. + the corresponding univalent) from different I—M groups; Figs. 14—15, I—A; Fig. 14, division of 2 univalents (distribution 6—2—6); Fig. 15, chromatin bridge and corresponding fragment. — Magnification: Figs. 11—13, 3375 ×, Figs. 14—15, 2250 ×.

presence of a fragment and a chromatin bridge (probably a double attachment chromatid), is represented in Fig. 15. The number of dividing univalents at I—A corresponds to the number of univalents found to be present at I—M. Consequently,

all or most of the univalents divide at I—A. This is quite in contrast to the fragment in the other *Aschersoniana* type described above. This fragment was never observed to divide at I—A. The reason of this different behaviour cannot be explained at present.

D. CORRELATION BETWEEN MEIOTIC BEHAVIOUR AND DEGREE OF POLLEN FERTILITY.

As already mentioned, the presence of an association of four chromosomes at meiosis in the *Aschersoniana* type just described is correlated with partial pollen sterility. In 1934 three different pollen samples studied (from two different clone plants) contained 52, 28 and 63 per cent good pollen respectively. In 1936 samples from three different clone plants gave the values 40, 40 and 60 per cent good pollen. The average of all values is 47 per cent. The plant may thus be regarded as semi-sterile like most other interchange heterozygotes. — This sterility is in accordance with the cytological observations. Of the rings and chains observed at I—M 26 were non-disjunctional, 38 in zig-zag arrangement. The percentage values of these two categories will be 40,6 and 59,4 respectively. The difference is $18{,}8 \pm 7{,}8$ and is thus probably significant. Since, presumably, the zig-zag arrangements give only viable gametes, the prevalence of such arrangements should slightly increase the amount of good pollen. However, as most of the I—M groups have only bivalents and half of the gametes from such groups will be unviable the total amount of bad pollen will be approximately 50 per cent, in spite of the prevalence of zig-zag arrangements.

In the type with a fragment, described under B, the percentage of apparently good pollen grains was examined six times in three different years. The following values were obtained: 90, 98, 98, 96, 93 and 94 per cent ($M = 94{,}8$). Thus, though the fragment is rather large it does not seem to influence pollen fertility at all. Neither could any reduction in the vigour of the plant be discerned. It is therefore very probable that the fragment represents a duplication of chromatin which is already present in one of the seven bivalents.

On an average pollen fertility in this individual with a fragment was equally good or even somewhat better than in the type first described (under A). In this type, having regular meiosis with the exception of occasional univalents, the following values were obtained (in three different years): 80, 95 and 91 per cent good pollen ($M = 88{,}7$).

At present I have ten different *Aschersoniana* types in culture, but of these only the three described above have been studied as to the mode of meiosis. Judging from the degree of pollen fertility, however, at least one of the remaining types may also show meiotic irregularities.

This type (No. 23) has on an average 64 per cent good pollen (average of six examinations) and may therefore be suspected to represent another interchange heterozygote. The remaining six biotypes had on an average the following percentages of good pollen: 84, 85, 92, 92, 95, 95. Most of these types will probably have regular meiosis though the presence of duplicated fragments is not excluded.

Meiotic irregularities in *D. Aschersoniana* have already been observed by KATTERMANN (1931). Besides biotypes with normal meiosis,

TABLE 1. *Chiasma frequencies in different biotypes of D. Aschersoniana.*

Field number and cytological category	Total number of chiasmata 6 7 8 9 10 11 12 13 14	n	M ± m	Chiasma number per chromosome
27, normal *Aschersoniana*	2 6 24 19 15 4 3	73	10,86 ± 0,15	0,776 ± 0,011
22, type with fragment ...	6 6 6 12 13 4 3	50	10,88 ± 0,24	0,777 ± 0,017
26, prep. 1, I-M groups without IV (generally 7_{II})	2 6 20 14 9 3	54	10,57 ± 0,16	0,755 ± 0,011
26, prep. 1, I-M groups with IV (or III)	2 6 8 14 8	38	11,53 ± 0,19	0,824 ± 0,014
26, prep. 1, total.....	2 8 26 22 23 11	92	10,97 ± 0,13	0,784 ± 0,009
26, prep. 1, bivalents in I-M groups with IV (or III)	2 5 9 15 7	38	8,53 ± 0,18	0,853 ± 0,018
26, prep. 2, I-M groups without IV (generally 7_{II})	1 — 1 1 10 13 20 6	52	11,23 ± 0,18	0,802 ± 0,013
26, prep. 2, I-M groups with IV (or III)	2 7 11 7 3	30	12,07 ± 0,20	0,862 ± 0,014
26, prep. 2, total.....	1 — 1 1 12 20 31 13 3	82	11,54 ± 0,14	0,824 ± 0,010
26, prep. 2, bivalents in I-M groups with IV (or III)	2 6 12 10	30	9,00 ± 0,17	0,900 ± 0,017

this author found a type in which one chromosome pair had a tendency to lie outside the equator and be eliminated. Further, the same author observed the occasional occurrence of $6_{II} + 2_{I}$ instead of 7_{II}.

E. CHIASMA FREQUENCIES IN D. ASCHERSONIANA.

At I—M the bivalents in *D. Aschersoniana* as well as in all other *Dactylis* types studied are generally united by one or two terminal chiasmata. As a rule there is no difficulty in distinguishing between the ring and the rod bivalents (cf. Fig. 1). The chiasma frequency was

counted in the three biotypes studied and the following result was obtained (Table 1).

As is evident from the table the average chiasma frequency per cell in the type having regular meiosis (No. 27) was $10{,}86 \pm 0{,}15$. This corresponds to an average chiasma frequency per chromosome of $0{,}776 \pm 0{,}011$. Hence, slightly more than half of the metaphase chromosomes in this type are united by two chiasmata, the rest by one. Almost exactly the same frequency was obtained in biotype No. 22 (type with fragment), the corresponding values being $10{,}88 \pm 0{,}24$ and $0{,}777 \pm 0{,}017$.

Of the third biotype, No. 26 (type with association of four chromosomes) two different slides were examined. In the first slide the average chiasma frequency did not differ significantly from that of the other two biotypes, the values found being $10{,}97 \pm 0{,}13$ and $0{,}784 \pm 0{,}009$. Owing to environmental influences of some kind (cf. MATHER, 1936) the chiasma frequency in the second slide was found to be significantly higher, the average values being $11{,}54 \pm 0{,}14$ and $0{,}824 \pm 0{,}010$ respectively.

When counting the chiasmata of this biotype it was observed that in cells containing an association of four chromosomes there seemed to be a prevalence of bivalents with two chiasmata. This observation was verified by a comparison between the chiasma frequencies in I—M groups with and without quadrivalents (Table 1). In preparation No. 1 the first category of cells had the average values $11{,}53 \pm 0{,}19$ and $0{,}824 \pm 0{,}014$, the corresponding values in the second category (groups without association of four) being $10{,}57 \pm 0{,}16$ and $0{,}755 \pm 0{,}011$. The difference is significant and shows that in this case *there is a positive correlation between the total chiasma frequency and the occurrence of quadrivalent associations*. The same positive correlation could also be observed in the second slide studied. In this slide I—M groups with only bivalents (or occasional univalents) had an average total chiasma frequency of $11{,}23 \pm 0{,}18$ per cell or $0{,}802 \pm 0{,}013$ per chromosome. In cells with associations of four (or occasionally three) chromosomes the corresponding values were $12{,}07 \pm 0{,}20$ and $0{,}862 \pm 0{,}014$. The difference $12{,}07 - 11{,}23$ is significant ($D = 0{,}81 \pm 0{,}27$). The difference in chiasma frequency in cells with and without quadrivalent associations probably depends entirely on the four chromosomes involved in the quadrivalents. If these four chromosomes fail to form a multiple association and are present as two pairs it is probable (on account of the structural change) that these bivalents will be rod-shaped. In such bivalents the chiasma number per chromosome will be 0,5. Now, the quadrivalents were generally open and seldom ring-shaped. In preparation No. 1, 33

quadrivalents appeared as chains (with 3 chiasmata) and only 3 as closed rings with 4 chiasmata. This gives an average chiasma number of 3,08 per association or 0,770 per chromosome. In the second slide the corresponding values were 3,15 and 0,787 respectively (22 chains and 5 rings). If the four chromosomes in question are present as rod-shaped bivalents and not as quadrivalents, this will evidently decrease the number of chiasmata per cell by about one unit ($3{,}08 - 2{,}00 = 1{,}08$ and $3{,}15 - 2{,}00 = 1{,}15$). In fact the differences observed between cells with and without quadrivalents were of about that size. As already mentioned, the difference in the first preparation was 0,96 and in the second slide 0,84. These values are slightly below expectation but this may be due to the fact that one or both of the bivalents in question may be capable occasionally of forming two chiasmata. This is indeed evident from Table 1, since in 9 cases of 106 the total chiasma number in cells without quadrivalents was as high as 13 (14 being the maximum number).

Further evidence has been obtained by counting the chiasma frequency of the five bivalents present in the same cells as the quadrivalent associations. If the chiasma frequency of these five bivalents is the same also in cells without quadrivalents the total chiasma frequency in such cells should be obtained by adding the chiasma number of the five bivalents and the chiasma number of the two bivalents consisting of structurally unequal partners. As inferred above, the number of chiasmata in the latter case should be 2,0 or a slightly higher value. Now, in the first slide the frequency of the five bivalents was found to be $8{,}53 \pm 0{,}18$ (Table 1) and the average value of complete groups without quadrivalents was equal to $10{,}57 \pm 0{,}16$. The latter value is very close to the expected figure ($8{,}53 + 2{,}0 = 10{,}53$). In the second slide a similar situation was met with. The average of the 5 bivalents was $9{,}00 \pm 0{,}17$ (Table 1) and the average of groups without quadrivalents $11{,}23 \pm 0{,}18$. The difference between the observed and expected values (11,23—11,00) is not significant.

Evidently the analysis of chiasma frequencies in this case strongly supports the evidence obtained from the meiotic chromosome configurations. In the plant in question four chromosomes are partially non-homologous. In some cases this results in associations of all four chromosomes at meiosis, but more frequently the chromosomes are united into two pairs. Owing to the structural dissimilarity between the members of these pairs the bivalents are generally rod-shaped, the chiasma frequency being definitely lower than in the other five pairs.

It is interesting, however, that the total average chiasma frequency in this *Aschersoniana* type is not lower than in the other two biotypes (27 and 22). This might be due to a negative correlation of chiasma frequencies, being an expression of a tendency to unitary control of the chiasma number per cell (cf. MATHER and LAMM, 1935; MATHER,

1936). — Even if the disturbing effects of environmental influences are taken into consideration (the difference between the total average values of Prep. 1 and Prep. 2 in biotype No. 26 is equal to $11{,}54 - 10{,}97 = 0{,}57 \pm 0{,}19$) it is clear that the chiasma frequency of the five bivalents in groups containing an association of four chromosomes (and of the corresponding five bivalents in groups without such associations) is higher than in the bivalents of the biotypes 27 and 22 (Table 1). The average frequencies per chromosome in these biotypes were $0{,}776 \pm 0{,}011$ and $0{,}777 \pm 0{,}017$ respectively. In biotype 26 the average chiasma frequency of the bivalent chromosomes, not involved in the quadrivalent association, was found to be $0{,}855 \pm 0{,}018$ (Prep. No. 1) and $0{,}930 \pm 0{,}017$ (Prep. No. 2). Both these values are significantly higher than the corresponding figures of biotypes 27 and 22. Consequently, the presence of two pairs of chromosomes in biotype 26, having a low chiasma frequency on account of incomplete homology, causes an increased chiasma frequency in the other bivalents and thanks to this compensation the total chiasma frequency will be approximately the same in all three biotypes and probably in most biotypes of the species. This negative correlation, however, is not strong enough to keep the total chiasma frequency of the individual cells constant, the metaphase groups containing an association of four chromosomes being observed to have a significantly higher frequency than groups without such associations. Negative correlations of chiasma frequencies were also observed in other *Dactylis* types (cf. below pp. 153—157).

2. MEIOSIS AND CHROMOSOME VARIATION IN DACTYLIS GLOMERATA.

A. CHROMOSOME NUMBERS IN PROGENIES OF D. GLOMERATA.

As already mentioned in the preliminary report (MÜNTZING, 1933 a), the chromosome number of *D. glomerata* is not quite stable. In addition to a majority of plants, having the typical number 28, a considerable proportion of aberrants with higher or lower numbers are formed. The data hitherto accumulated are given in Table 2. The four progenies in the upper part of the table (33—10, 35—21, 32—018 and 34—13) were all raised from single isolated mother plants, verified to have $2n = 28$. The other four progenies (34—14, 34—15, 38→70 and 72→107) were raised from seeds kindly sent to me by Mr. K. FLOVIK (Norway) and Dr. O. VALLE (Finland). Though this seed was probably produced by open pollinated plants and the chromosome numbers of the mother plants were not determined, chromosome variation in these

progenies is evidently of the same type as in the other progenies from controlled mother plants.

For determination of the chromosome numbers root tips were fixed in chrom-acetic-formalin and the sections stained with gentian violet. The fixations were on an average good, but sometimes it was difficult to count the number with absolute accuracy [1]. Therefore I have found it necessary to distinguish between »total» and »accurate» values. A

TABLE 2. *Chromosome numbers in progenies of Dactylis glomerata.*

Field number	Chromosome number: 26	27	28	29	30	n	Percentage of aberrants
33—10, (Skandia II) isol., acc. v.		1	22	1		24	8
» » » » total		4	30	1		35	14
35—21, » » » acc. v.		3	19			22	14
» » » » total	1	6	32	3	1	43	26
32—018, accurate (= total) values		3	21	3	1	28	25
34—13 (*Dactylis* Altai), acc. v.		1	25	2		28	11
» » » total	2	5	33	3		43	23
34—14 (*Dactylis* Norway), acc. v.			8			8	0
» » » total		2	14	5		21	33
34—15 (*Dactylis* Norway), acc. v.		1	7			8	13
» » » total		1	7	2		10	30
38→70 (*Dactylis* Finland), acc. v.			28	1		29	3
» » » total			27	1		28	4
72→107 (*Dactylis* Finland), acc. v.		1	29			30	3
» » » total		1	24			25	4
Sum of all accurate values		10	159	7	1	177	10
» » » total »	3	22	188	18	2	233	19

chromosome number was considered to be accurately determined when at least three different somatic plates (first drawn and then counted) gave the same number. On the other hand, if for instance four plates were counted, giving the numbers 29, 28, 29, 29, the chromosome number in this case would be given as ± 29. The »total» values in the table include both the accurate values and the ± values. Considering the accurate values (Table 2) the total percentage of aberrants was found to be 10 (159 plants with 2n = 28, 18 plants with 27, 29 or 30 chromo-

[1] The determination of the somatic chromosome numbers has chiefly been performed by different persons working in the Cyto-genetic Laboratory at Svalöf. I wish to express my sincere gratitude for this valuable cooperation.

somes). This is a minimum value. On basis of the total values the corresponding figure will be as high as 19 per cent (45 aberrants among 233 plants examined). The true percentage of aberrants has probably a value intermediate between 10 and 19, as the exclusion of all ± values will probably tend to decrease the real percentage of aberrants. For example, judging from vigour and fertility, the three plants having $2n = \pm 26$ (Table 2) must be real aberrants although their chromosome number could not be exactly determined. The same is true of several other ± aberrants. It seems probable that the same correlation exists between plant vigour and the result of fixation in *Dactylis* as in *Phleum* (MÜNTZING, 1935). If that is the case this will tend to decrease the percentage of aberrants accurately determined, since the aberrants in *Dactylis* are on an average less vigorous than the euploid plants with 28 chromosomes (cf. below). — Hence, although the percentage of aberrants cannot be given accurately, this does not influence the main conclusion, viz. that *D. glomerata is cytologically unstable, aberrants with deviating chromosome numbers being present in every progeny tested.*

B. MEIOSIS IN DACTYLIS GLOMERATA.

As already demonstrated (MÜNTZING, 1933 a) the formation of aberrants in *D. glomerata* is ultimately caused by the presence of quadrivalents at meiosis (Figs. 16—20). About half of the chromosomes are united to quadrivalents of different types, which are chiefly arranged in a zig-zag fashion. Although disjunction at I—A will mostly be regular, exceptions to this rule will lead to formation of gametes with deviating chromosome numbers and hence to aberrants in the progeny. — Meiosis in *D. glomerata* was also studied by RANCKEN (1934), who in the main confirmed the previous observations. This author, however, also found trivalents and univalents to be rather frequent at I—M and presents evidence of secondary association.

Since 1933 the study of meiosis in *D. glomerata* has been somewhat extended. Fuller information has been acquired as regards meiosis in the commercial variety »Skandia II» (the original clone) and additional data have been obtained from a study of a wild *glomerata* type from Altai (originally collected by Prof. G. TURESSON). The following results have been obtained [1]:

a. *Diakinesis and first metaphase.* — In Skandia II the following configurations were observed in 50 complete I—M groups:

[1] In this account the repetition of some data already reported cannot be avoided.

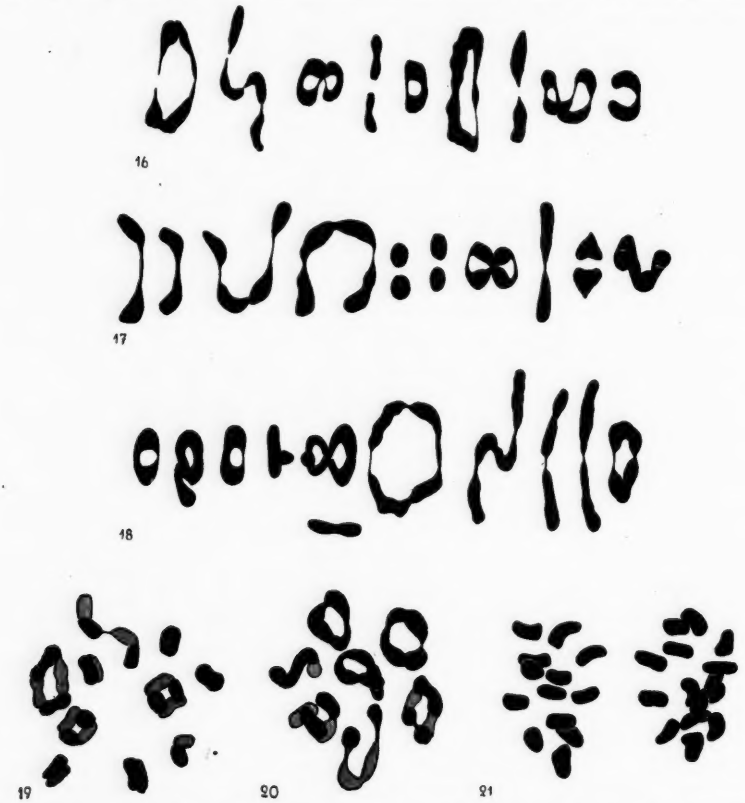
Configuration	Side view	Polar view	Total
14_{II}	0	0	0
$1_{IV} + 12_{II}$	1	2	3
$2_{IV} + 10_{II}$	7	2	9
$3_{IV} + 8_{II}$	3	10	13
$4_{IV} + 6_{II}$	4	8	12
$5_{IV} + 4_{II}$	2	6	8
$6_{IV} + 2_{II}$	0	1	1
7_{IV}	0	2	2
$1_{IV} + 1_{III} + 9_{II} + 3_{I}$	1	0	1
$3_{IV} + 1_{III} + 6_{II} + 1_{I}$	1	0	1
Average number of IV (+ III):	2,95	3,81	3,48

As is evident from the table the presence of trivalents + univalents instead of quadrivalents is quite rare in the present material. An exceptional group of this kind is represented in Fig. 18 ($3_{IV} + 1_{III} + 6_{II} + 1_{I}$). All the possible combinations of quadrivalents and bivalents have been observed and in two cases the maximum association, 7_{IV} (Fig. 20). The only expected configuration lacking was 14_{II}. Every one of the 50 cells examined contained at least one quadrivalent. The lower average frequency of quadrivalents in side view than in polar view is probably due to observational difficulties, groups with a relatively low number of IV:s being easier to analyse and therefore unconsciously selected. The total average, 3,48, must consequently be regarded as a minimum value. At any rate, *at least half of the chromosomes of this biotype are on an average united to quadrivalents.*

The occurrence of quadrivalents is not restricted to the cultivated biotypes of *D. glomerata* but is probably characteristic of all biotypes of the species. This view is strongly supported by the *frequent occurrence of quadrivalents also in the absolutely wild biotype from Altai.* In this biotype diakinesis and first metaphase could be studied. At diakinesis (Figs. 22—23) the following configurations were found: $1_{IV} + 12_{II}$ (5 cells), $2_{IV} + 10_{II}$ (4 cells), $3_{IV} + 8_{II}$ (7 cells), $4_{IV} + 6_{II}$ (7 cells), $5_{IV} + 4_{II}$ (1 cell) and 7_{IV} (1 cell). Thus, the number of quadrivalents in the 25 cells studied varies from 1 to 7 and is on an average 3,0. It is noteworthy that also in this biotype the maximal association 7_{IV} was observed (Fig. 23) and that this configuration occurred at diakinesis. The chromosomes of the quadrivalent groups are evidently united by chiasmata and not only by secondary association. — Some additional chromosome counts could be made at first metaphase of the same biotype. The following configurations were observed (from polar and side views): $1_{IV} + 12_{II}$ (2 cells), $2_{IV} + 10_{II}$ (4 cells), $3_{IV} + 8_{II}$ (1 cell) and $4_{IV} + 6_{II}$ (5 cells). The average frequency of quadrivalents in these 12 cells is 2,8. It seems probable that the frequency of quadrivalents is slightly lower in the wild biotype from Altai than in the commercial variety studied, but the data hitherto gathered are not sufficient to prove that definitely. It is certain, however, that quadrivalents are frequent in both biotypes, and the maximum association 7_{IV} has been observed in both of them.

Concerning the types of quadrivalents and the arrangement of the chromosomes in these associations the following details may be given: In the main there are four types of quadrivalents, *zig-zag rings* (e. g. Fig. 16, 3rd and 8th configuration from the left, Fig. 17, 4th from the right), *non-zig-zag rings* (e. g. Fig. 16, 1st and 6th configuration from the left), *zig-zag chains* (e. g. Fig. 16, 2nd configuration from

the left, Fig. 18, 4th from the right) and *non-zig-zag chains* (e. g. Fig. 17, 3rd and 4th from the left). These different types may also be distinguished in polar views. In Fig. 19, for instance, there are three zig-zag rings and one zig-zag chain. — Of 52 quadrivalents observed in side view 25 were zig-zag rings, 12 zig-zag chains, 11 non-zig-zag rings and 4 non-zig-zag chains. Consequently, most of the configurations



Figs. 16—21. Meiosis in *Dactylis glomerata* (»Skandia II»). Figs. 16—18, I—M, side view (separately drawn); Fig. 16, $5_{IV} + 4_{II}$; Fig. 17, $4_{IV} + 6_{II}$; Fig. 18, $3_{IV} + 1_{III} + 6_{II} + 1_{I}$; Figs. 19—20, I—M, polar view; Fig. 19, $4_{IV} + 6_{II}$; Fig. 20, 7_{IV}; Fig. 21, I—A (distribution 13—15). — Magnification: 3375 ×.

(about 70 per cent) appear as closed rings, but a considerable proportion (30 per cent) as chains. Further, *in the quadrivalents there is a marked tendency for adjacent chromosomes to go to opposite poles*, about 70 per cent of the quadrivalents being arranged in zig-zag. This is about the same figure as previously reported for 67 quadrivalents observed in polar view (MÜNTZING, 1933 a, p. 204).

b. *First anaphase and interphase.* — In »Skandia II» the chromosome dis-

tribution of 52 cells at I—A was observed. The following types and frequencies of distribution occurred: 14—14: 44 cells; 13—15: 2 cells (Fig. 21); 13—1—14 (one chromosome between the anaphase groups): 4 cells; 13—2—13 (two chromosomes between the groups): 2 cells. — In »*Dactylis* Altai» only 13 I—A groups could be counted, all of them showing the distribution 14—14. — These data suffice to show that *most gametes* (at least on the male side) *will receive 14 chromosomes, but it is also certain that a low frequency of gametes with deviating chromosome numbers will be formed.* — In both biotypes studied there was a low frequency of univalents dividing at I—A. In »Skandia II» 95 cells were without dividing univalents, in 3 cells there was one and in 2 cells two dividing univalents, the total percentage of cells with dividing univalents thus being as low as 5. In »*Dactylis* Altai» the percentage was found to be 4, the corresponding values being 96, 2 and 2. — In the latter biotype the frequency of micronuclei at interphase was counted. Of a hundred cells at this stage only 5 contained one micronucleus and in one cell there were two micronuclei. — Later stages have not been examined but they are of little interest for the present problem. The data given suffice to show that the *glomerata* aberrants with deviating chromosome numbers are produced on account of slight meiotic irregularities.

C. MEIOSIS IN SOME GLOMERATA ABERRANTS.

a. *A trisomic glomerata* ($2n = 27$). — Meiosis was studied in a *glomerata* aberrant (D. 6) with 27 instead of 28 chromosomes. In the main the meiotic picture was of the same type as in pure *glomerata*, i. e. quadrivalents were frequent. The lack of one chromosome, however, caused a rather frequent occurrence of trivalents and univalents in addition to the quadrivalents.

At first metaphase 20 complete cells (in side view) were found to contain the following configurations:

a) Configurations without trivalents

$1_{IV} + 11_{II} + 1_I$: 1 cell
$2_{IV} + 9_{II} + 1_I$: 3 cells
$3_{IV} + 7_{II} + 1_I$: 2 »
$4_{IV} + 5_{II} + 1_I$: 2 »
$5_{IV} + 3_{II} + 1_I$: 4 »
$6_{IV} + 1_{II} + 1_I$: 1 cell

b) Configurations with trivalents

$1_{IV} + 1_{III} + 10_{II}$: 1 cell
$3_{IV} + 1_{III} + 6_{II}$: 2 cells
$5_{IV} + 1_{III} + 2_{II}$: 1 cell
$1_{IV} + 1_{III} + 9_{II} + 2_I$: 1 »
$2_{IV} + 1_{III} + 7_{II} + 2_I$: 1 »
$1_{IV} + 2_{III} + 7_{II} + 3_I$: 1 »

In 13 cases out of 20 the group of three chromosomes, homologous to the missing one, evidently appeared as $1_{II} + 1_I$ (Fig. 24), and in at least 4 of the remaining seven cases these chromosomes form a trivalent (Fig. 25). In three cells, containing both trivalents and univalents, the interpretation is less certain. It is probable, however, that also in these cases the trivalent (or one of the two trivalents) is formed by the three chromosomes in question and the univalents by non-conjunction in one pair.

The average number of quadrivalents in these cells is 3,15 and the average number of multiple configurations (IV + III) is *3,55*. Though the present plant is not related to the biotypes of pure *glomerata* examined, the latter figure agrees very well with the average number of quadrivalents found in these biotypes. The absence of

one chromosome in this case has evidently no effect on the association in the other chromosome groups. — As to the type and frequency of the quadrivalents the following numbers were noted: 17 zig-zag rings, 22 non-zig-zag rings, 14 zig-zag chains and 10 non-zig-zag chains. As before, rings are more frequent than chains but in contrast

Figs. 22—27. Meiosis in *Dactylis glomerata* (continued). — Figs. 22—23, diakinesis in a biotype from Altai; Fig. 22, $3_{IV} + 8_{II}$; Fig. 23, 7_{IV}. — Figs. 24—25, I—M groups (side view, separately drawn) from an aberrant plant with $2n = 27$; Fig. 24, $5_{IV} + 3_{II} + 1_{I}$; Fig. 25, $1_{IV} + 1_{III} + 10_{II}$; Fig. 26, I—A from the same plant (division of one univalent, distribution 13—2/2—13). — Fig. 27, I—A (polar view, separately drawn) from a plant with 29 chromosomes (»030—1/5»), distribution 14—15. — Magnification: 3375 ×.

to »Skandia II» the present plant does not show any prevalence of zig-zag-arrangements.

As might be expected from the types of I—M configurations division of one univalent was frequent at I—A. In a few cases 2—3 univalents were observed to divide. The following frequencies were noted:

Number of dividing univalents ..... :	0	1	2	3
» » cells ..... :	14	33	3	2

In 15 cases the total chromosome number at I—A could be distinguished. The following distributions were observed: 13—14 (7 cells), 13—1—13 (4 cells), 12—1—14 (2 cells), 12—2—13 (1 cell) and 12—3—12 (1 cell). Some of the cells showing 13—14 distribution were in quite early anaphase and might therefore, if observed later, be found to give lagging chromosomes. 13—1—13 is probably the most common distribution (Fig. 26).

A few cells at interphase were observed to contain eliminated chromosomes outside the interphase nuclei. No doubt, in this plant, as in most cases where univalents are present, a certain amount of chromosome elimination occurs. Therefore, at least in the pollen, *the gametes will more often contain 13 than 14 chromosomes.*

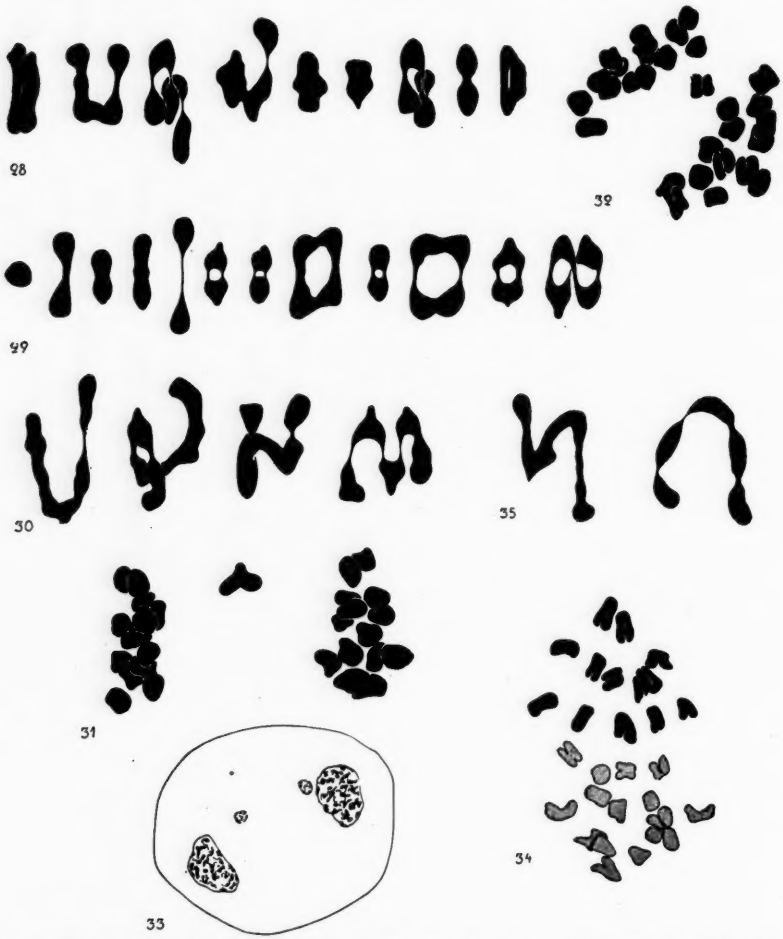
b. *Pentasomic glomerata types* ($2n = 29$). — Meiosis has been studied in two different *glomerata* plants, having one chromosome more than typical *glomerata* (Figs. 27—33). The behaviour of the extra chromosomes in these two plants was found to be significantly different.

Taking plant No. 030—1/5 (mother plant of the progeny 32—017, Table 3) into consideration first the following I—M configurations (chiefly side views) were found in 21 cells examined:

Configuration	Number of cells	Configuration	Number of cells
1) $1_V + 1_{IV} + 10_{II}$	: 2	7) $1_{IV} + 2_{III} + 9_{II} + 1_I$	: 1
2) $1_V + 1_{IV} + 1_{III} + 8_{II} + 1_I$	: 2	8) $2_{IV} + 1_{III} + 9_{II}$	: 2
3) $1_V + 2_{IV} + 1_{III} + 6_{II} + 1_I$	: 1	9) $4_{IV} + 1_{III} + 5_{II}$	: 1
4) $1_V + 3_{IV} + 6_{II}$	: 2	10) $3_{IV} + 8_{II} + 1_I$	: 3
5) $1_V + 4_{IV} + 4_{II}$	: 1	11) $4_{IV} + 5_{II} + 3_I$	: 1
6) $1_V + 5_{IV} + 2_{II}$	: 2	12) $5_{IV} + 4_{II} + 1_I$	: 3

In about half of the cells the extra chromosome is evidently present as a member of a quinquevalent (Fig. 28), in configurations Nos. 7—9 it is responsible for the formation of trivalents, while in configurations Nos. 10—12 it is unpaired. — As in the other *glomerata* types, quadrivalents are frequent, the average value being *3,5* per cell (quinquevalents also included). The four usual types of quadrivalents were met with in the following frequencies: 18 zig-zag rings, 11 straight rings, 8 zig-zag chains and 15 non-zig-zag chains. In addition to these a new type of quadrivalent was met with twice. This type (Fig. 28, fourth configuration from the left) may be described as a ring bivalent with a rod bivalent attached at one side or as a »frying pan» configuration with two chromosomes in the arm. — It is obvious that in the quadrivalents of this *glomerata* type zig-zag and non-zig-zag arrangements are equally frequent. Of the nine quinquevalents observed in side view five were W-shaped (Fig. 30, first and second from the right), one was straight (Fig. 30, first from the left) and three were partly zig-zag shaped, partly straight (Fig. 30, 2nd from the left).

Judging from the 21 I—M groups completely analysed (cf. above), about half of the cells at this stage contain 1—3 univalents. For comparison with the other pentasomic plant described below, the number of univalents, lying outside the I—M



Figs. 28—35. Meiosis in *Dactylis glomerata* (continued). — Figs. 28—33, divisions from pentasomic plants ($2n = 29$); Figs. 28—29, I—M (side view, separately drawn); Fig. 28, $1_V + 4_{IV} + 4_{II}$; Fig. 29, $3_{IV} + 8_{II} + 1_I$; Fig. 30, four separate quinquevalents (I—M); Figs. 31—32, I—A, distribution 14—1—14; Fig. 33, interphase with micronuclei. — Fig. 34, I—A from an aberrant with $2n = 30$, distribution 13—17; Fig. 35, two separate quinquevalents from the same plant (I—M). — Magnification: 1688 × in Fig. 33, 3375 × in the other figures.

groups, was counted in two different slides from different fixations. The following numbers were obtained:

Slide No.	0	1	2	3	univalents
1:	79	29	1	—	cells
2:	71	36	13	1	»
Total:	150	65	14	1	»

The values correspond to 65 per cent of cells with no univalents visible, 28 per cent with one, 6 per cent with 2 and less than one per cent with three univalents outside the I—M groups.

Most of the univalents seem to divide at I—A. In the two slides examined the following numbers were counted:

Slide No.	0	1	2	univalents dividing
1:	84	33	3	cells
2:	55	23	4	»
Total:	139	56	7	»

These values correspond to 69 per cent of cells without dividing univalents, 28 per cent with division of one and 3 per cent with division of two univalents.

Only a limited number of complete I—A groups could be counted, but in 12 cells the distribution was found to be 14—15 (Fig. 27) and in 2 cells 14—1—14 (Fig. 31). — The chromosomes lagging and dividing at I—A are evidently often eliminated (Fig. 33). Of 100 cells at interphase 30 contained micronuclei and 70 were without. At second anaphase real lagging chromosomes were quite rare and were only observed in 5 per cent of 152 cells examined, but on the other hand chromosomes, evidently eliminated in the first division, were frequently seen.

From the observations described above it is evident that in the first pentasomic *glomerata* plant studied less than half of the gametes will receive the extra chromosome. The ratio of gametes with 14 and 15 chromosomes may be estimated to be about 65 : 35.

In the second pentasomic *glomerata* type (D. 8) the extra chromosome is evidently eliminated in a still higher degree. This is due to the fact that in this plant the extra chromosome is usually present as a univalent and seldom as a member of trivalents or quinquevalents.

Only six I—M groups could be completely analysed, but these were found to have the following constitutions (one cell of each kind); $2_{IV} + 10_{II} + 1_I$, $3_{IV} + 1_{III} + 6_{II} + 2_I$, $3_{IV} + 1_{III} + 7_{II}$, $3_{IV} + 8_{II} + 1_I$ (Fig. 29), $4_{IV} + 6_{II} + 1_I$ and $5_{IV} + 4_{II} + 1_I$.

The number of univalents outside the I—M groups was counted in two slides from different fixations. The following numbers were observed:

Slide No.	0	1	2	3	univalents
1:	16	36	4	1	cells
2:	38	59	6	1	»
Total:	54	95	10	2	»

These values correspond to 34 per cent of cells with no univalents visible, 59 per cent with one, 6 per cent with two and 1 per cent with three univalents outside

the I—M groups. — In the previous pentasomic type described the corresponding values were 65, 28, 6 and 1. *There is evidently a significant difference between the two plants as regards the relative frequency of cells with 0 or 1 univalent.* — There is also a significant difference with regard to the number of univalents dividing at I—A. In the previous pentasomic type 69 per cent of the cells contained no dividing univalents, 28 per cent had one and 3 per cent two dividing univalents. In the present type the number of cells with one dividing univalent was much higher. The following values were observed in two slides:

Slide No.	0	1	2	3	univalents dividing
1:	51	67	9	2	cells
2:	34	54	2	1	»
Total:	85	121	11	3	»
Per cent:	39	55	5	1	

From the data given above and from the observation that lagging and dividing univalents are in most cases eliminated, it is evident that *most gametes of D. 8 will have 14 chromosomes.* About 17 per cent may contain 15 chromosomes and a still lower percentage will receive still more deviating chromosome numbers. The occasional formation of gametes with other numbers than 14 and 15 is evident from some chromosome counts at I—A. Nine cells examined showed the following distributions: 14—1—14 (3 cells), 15—14 (2 cells), 15—1—13 (1 cell) and 16—13 (3 cells). One of the anaphases showing 16—13 distribution, however, was quite early and might in reality contain a lagging univalent.

c. *Observations from plants with 30 chromosomes.* — A few scattered observations were made concerning the mode of meiosis in two plants with $2n = 30$. These individuals were both daughters of the pentasomic plant described above (030—1/5) and belong to the progeny 32—017 (Table 3). A few I—M groups could be analysed with some difficulty and were found to contain the following configurations: $2_{IV} + 3_{III} + 6_{II} + 1_I$ (2 cells), $3_{IV} + 1_{III} + 7_{II} + 1_I$ (1 cell), $4_{IV} + 1_{III} + 5_{II} + 1_I$ (1 cell) and $5_{IV} + 1_{III} + 3_{II} + 1_I$ (1 cell). Quinquevalents (Fig. 35) were observed in other cells not completely analysed, but no hexivalents could be detected. As the plants in question are daughters of a pentasomic individual they may be expected to be $4n + 2$ forms rather than $4n + 1 + 1$.

In both plants 1 or 2 univalents were sometimes present at I—M. Of 136 cells examined 60 per cent were without visible univalents, 32 per cent had one and 7 per cent two univalents. Finally, it should be mentioned that a cell at I—A was observed to have the distribution 13—17 (Fig. 34). Thus, as might be expected, these individuals in spite of their even chromosome number were found to have a rather irregular meiosis, resulting in gametes with several different chromosome numbers.

D. CHROMOSOME VARIATION IN PROGENIES OF GLOMERATA ABERRANTS.

As already exemplified in the preliminary report (MÜNTZING, 1933 a) a marked variation in chromosome number may be obtained by raising progenies from *glomerata* aberrants. Thus, in the progeny from a plant with $2n = 29$ the chromosome numbers varied from 27 to 31. This progeny is included in Table 3 under the number of 32—017. In the same table the chromosome variation in eight other progenies is given.

The mother plants of these progenies have 27, 29 or 30 chromosomes. In this case, too, it seemed desirable to distinguish between »accurate» and »total» values. Without exception, however, the two series formed by these values are of exactly the same type and give about the same means.

A general feature is strikingly evident when a comparison is made between the chromosome numbers of the mother plants and the average

TABLE 3. *Chromosome numbers in progenies from aberrants of Dactylis glomerata.*

Field number	Chromosome number of the mother plant	Total or accurate values	Chromosome numbers in the progeny 26 27 28 29 30 31 32	n	M
D. 2	27	accurate	7	7	27,0
»	»	total	8	8	27,0
D. 4	27	accurate	10 9	19	27,5
»	»	total	5 20 17 — 1	43	27,4
D. 6	27	accurate	1 1 7 2 1	12	28,1
»	»	total	1 1 8 3 1	14	28,1
D. 7	29	accurate	2 9 1 2 1	15	28,4
»	»	total	5 18 6 7 2	38	28,6
D. 8	29	accurate	1 6 17 6 1	31	28,0
»	»	total	3 10 19 10 2	44	28,0
32—017	29	all values accurate	2 29 30 14 1	76	28,8
D. 9	30	accurate	1 6 10 2 1	20	29,8
»	»	total	2 10 18 8 1	39	29,9
017—22, a	30	accurate	2 — 1	3	29,7
»	»	total	1 4 — 1	6	29,2
017—22, b	30	accurate	1 13 31 9 — 1	55	29,0
»	»	total	2 15 37 10 — 1	65	28,9

values of their progenies. *In the progenies there is a marked tendency to revert to the number 28, the typical chromosome number of D. glomerata.*

Taking the progenies from mother plants with $2n = 27$ first, we find that the average (total) values were 27,0, 27,4 and 28,1 respectively. Meiosis was studied in the mother plant (D. 6) of the last-mentioned progeny (cf. above pp. 127—129). Owing to the elimination of univalents it was concluded that the gametes of this plant (at least in the pollen) would more often contain 13 than 14 chromosomes. In the absence of selective processes this would lead to a majority of plants with $2n = 26$, a lower number with $2n = 27$ and a still lower number

with $2n = 28$. However, most of the daughter plants examined had 28 chromosomes or higher numbers, only one plant with 26 and one with 27 chromosomes being represented in the progeny. In the other two progenies of individuals with $2n = 27$ the average values are somewhat lower but are nevertheless much higher than those expected if all gametes and zygotes were equally viable. As univalents are probably present in all plants with 27 chromosomes and as at least part of these univalents are eliminated, the average values might be expected to be always lower than 27 and not higher. The opposite result shows that there must be a rather marked selection of gametes or zygotes (or both), tending to regulate the chromosome number back to 28.

Though the number of individuals is relatively low, a calculation of the standard errors showed that the progenies of D. 4 and D. 6 most probably represent different distributions. If the average variance of the two series is used for the calculation of the standard errors, D. 6 will have the average value $28{,}14 \pm 0{,}22$, the corresponding figure of D. 4 being $27{,}35 \pm 0{,}13$. The difference will be $0{,}79 \pm 0{,}25$ and is significant. If the variance and standard errors of each series are calculated separately the standard error of the difference will be slightly higher (0,28), but even in this case the difference may be regarded as significant.

The difference between these two progenies may be due to the absence of two different chromosomes in the mother individuals, the lack of the chromosome in D. 6 being more injurious than in D. 4. This conclusion is strongly supported by the fact that D. 4 is a much more vigorous plant than D. 6. Anticipating in this case the discussion of the correlation between chromosome number and vigour, it may be mentioned that the two plants were weighed in three different years (as single plants or as clone plants). The average values of D. 4 were 530, 870 and 207 gr. respectively, the exactly corresponding values of D. 6 being 360, 80 and 23 gr. The height of the two plants also showed a similar difference. With respect to pollen fertility, however, no difference could be detected, both plants having more than 90 per cent apparently good pollen.

In contrast to the progenies from mother individuals with 27 chromosomes the progenies raised from pentasomic plants ($2n = 29$) show a decrease in the average chromosome number, the mean values being 28,6, 28,0 and 28,8 respectively (Table 3). Of the three series, however, the progeny of D. 8 has markedly lower values than the other series. The contrast between the D. 8 and 32—017 series is especially marked and was found to be statistically significant, the difference between the

two average values having the value $0{,}83 \pm 0{,}17$ ($D/m = 4{,}9$). — This difference is at once comprehensible, when it is recalled that meiosis in the two mother plants was found to be significantly different (cf. above pp. 129—132). In D. 8 the extra chromosome was in most cases present as a univalent and therefore eliminated, the frequency of gametes with 15 chromosomes probably being about 17 per cent. As most of the other gametes will contain 14 chromosomes, the majority of the daughter plants must in fact have 28 chromosomes as observed. The low frequency of gametes with 15 chromosomes and other still more aberrant numbers accounts for the occurrence of plants with 26, 27, 29 and 30 chromosomes. In this case the chromosome distribution at meiosis is completely in accordance with the type of progeny obtained, and there is no need to assume any selective influences.

The same thing is true of 32—017. The higher average chromosome number in this progeny is no doubt caused by the lower frequency of univalents and in consequence the lower frequency of meiotic elimination in this case. The extra chromosome in the mother individual (030—1/5) was in most cases a member of a trivalent or quinquevalent, and the average percentage of gametes with 15 chromosomes was estimated at about 35. Disregarding the low frequency of gametes with 13 chromosomes, the proportion of gametes with 14 and 15 chromosomes will approximately be as 65 : 35. In such a case the ratio of individuals in the progeny with 28, 29 and 30 chromosomes will be as 4225 : 4550 : 1225. These values agree well with those actually obtained, *viz.* 29, 30 and 14. This agreement again indicates that there is not much selection among the gametes and zygotes. The relatively high number of 30-chromosome plants also strongly indicates that pollen grains with 15 chromosomes are quite functional.

Of the progenies from 30-chromosome mother individuals the one from D. 9 is remarkable by showing only a very slight decrease of the average chromosome number (cf. Table 3), plants with 31 and 32 chromosomes being formed in about the same frequency as individuals with $2n = 29$. Though meiosis in the mother plant has not yet been studied, it may safely be concluded that not only female but also male gametes with more than 14 chromosomes frequently function. Further, it should be mentioned already here that on an average this progeny has a quite good viability, even plants with more than 30 chromosomes being quite vigorous.

The progeny 017—22 a (Table 3) only comprises 6 individuals, five of which have a lower chromosome number than the mother plant. The

progeny 017—22 b is from the same mother plant, but the seeds in this case were harvested after open pollination (all the other progenies are from isolated mother plants). As the mother plant was chiefly surrounded by normal *glomerata* plants with 28 chromosomes, it is probable that most of the functional male gametes in this case had 14 chromosomes. In such a case most of the female gametes had 15 chromosomes but also lower and higher numbers.

Conclusion. — From the data presented above it is evident that in progenies from *glomerata* aberrants there is a general tendency to revert to the normal chromosome number of *glomerata* ($2n = 28$). This tendency, however, is more or less pronounced in different progenies and depends partly on the quality of the particular chromosome involved, partly on the mode of meiosis. In some cases extra chromosomes are eliminated more frequently than in other cases. The reversion to 28 from plants with lower numbers must be due to the selection of gametes or zygotes, the reversion to 28 from plants with higher numbers is chiefly due to the elimination of univalents at meiosis. Even male gametes with extra chromosomes are functional.

3. MEIOSIS IN TRIPLOID GLOMERATA × ASCHERSONIANA HYBRIDS.

No artificial hybrids between the two *Dactylis* species have been produced but spontaneous hybrids have been obtained from two different localities near Svalöf. At these localities (discovered by Dr. N. SYLVÉN) the two species were growing in close proximity. Hybrid individuals were picked out partly by morphological inspection, partly by a microscopical examination of the quality of the pollen. By means of the latter method in particular a number of hybrids (12 in all) were obtained without difficulty. These hybrids have 21 chromosomes (Fig. 36) and are all completely male sterile. These plants can certainly be regarded as F_1 hybrids, since they have exactly 21 chromosomes and are morphologically intermediate between *D. Aschersoniana* and *glomerata* (cf. below p. 192).

Meiosis in the p. m. c. of these hybrids is characterized by a high frequency of trivalents (Figs. 37—41) and a marked degree of chromosome elimination (Figs. 44—53). The average number of trivalents was found to be $4{,}58 \pm 0{,}20$ and consequently about 65 per cent of the chromosomes in these hybrids are united to trivalents. In order to facilitate the study of meiosis in other *Dactylis* types with higher chromosome numbers, meiosis in the triploid hybrids was studied in detail. The following observations were made:

A. FIRST METAPHASE CONFIGURATIONS.

7_{III} 4 cells	$3_{III} + 4_{II} + 4_{I}$: 10 cells
$6_{III} + 1_{II} + 1_{I}$: 12 »	$2_{III} + 5_{II} + 5_{I}$: 1 cell
$5_{III} + 2_{II} + 2_{I}$: 12 »	$1_{III} + 6_{II} + 6_{I}$: 2 cells
$4_{III} + 3_{II} + 3_{I}$: 11 »	$5_{III} + 1_{II} + 4_{I}$: 1 cell

Of these 53 complete I—M groups 50 were observed in side view, 3 in polar view. The number of trivalents varies from 1 to 7 and is on an average 4,58 ± 0,20. With

Figs. 36—43. Chromosomes from spontaneous hybrids between *D. glomerata* and *Aschersoniana*. — Fig. 36, somatic plate ($2n = 21$); Figs. 37—39, I—M, side view, separately drawn, Fig. 37, 7_{III}; Fig. 38, $6_{III} + 1_{II} + 1_{I}$; Fig. 39, $4_{III} + 3_{II} + 3_{I}$; Figs. 40—41, I—M, polar view; Fig. 40, $4_{III} + 3_{II} + 3_{I}$; Fig. 41, 7_{III}; Figs. 42—43, I—A; Fig. 42, distribution 10—11; Fig. 43, division of five univalents, distribution 7—5—9. — Magnification: 3375 ×.

the exception of one single case ($5_{III} + 1_{II} + 4_I$), the number of bivalents and univalents is always the same, and the sum of the trivalents and bivalents (or univalents) is always seven. *The chromosome complement consequently consists of seven sets of three homologous or mainly homologous chromosomes, which are capable of giving a maximum association of 7 trivalents* (Figs. 37 and 41).

Four types of trivalents were observed. Of a total of 225 trivalents examined 147 (65 per cent) were V-shaped (e. g. all trivalents in Fig. 38), 42 (19 per cent) were of the »frying pan» type (Fig. 39, third trivalent from the left), 33 (15 per cent) were Y-shaped (Fig. 39, first trivalent from the left) and 3 (1 per cent) appeared as straight chains (———). All chiasmata at this stage were terminal. No trivalents of the triple arc type were seen.

B. FIRST ANAPHASE.

I—A is generally characterized by the presence of a variable number of dividing univalents. In 104 cells examined the average number of dividing univalents was found to be $2{,}59 \pm 0{,}11$, the distribution being as follows:

Number of dividing univalents	0	1	2	3	4	5
» » cells	2	17	29	34	18	4

Complete chromosome counts in 38 cells gave the following I—A distributions: 9—2—10 (8 cells), 8—3—10 (8 cells), 9—3—9 (6 cells), 11—1—9 (4 cells), 10—1—10 (3 cells), 7—4—10 (3 cells), 8—2—11 (2 cells), 9—4—8 (1 cell), 7—5—9 (1 cell, Fig. 43), 8—1—12 (1 cell), 10—11 (3 cells, Fig. 42), 9—12 (1 cell) and 8—13 (1 cell).

C. FIRST TELOPHASE, INTERPHASE AND SECOND METAPHASE.

A large proportion of the univalents dividing at I—A evidently never reach the poles in time to be included in the interphase nuclei, and in some of the lagging univalents not even the division is completed (Figs. 44—46). These eliminated bodies are also frequent at second metaphase (Fig. 50). The number of eliminated bodies outside the interphase nuclei and outside the second metaphase groups was counted with the following result:

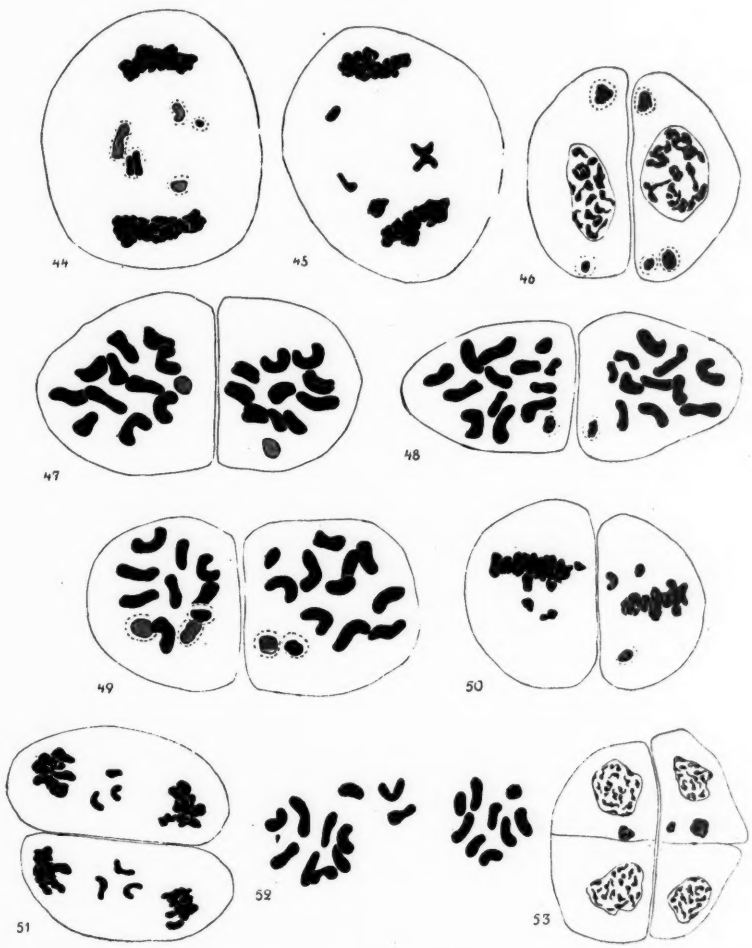
Number of eliminated bodies:	0	1	2	3	4	5	6	7	8	n	M ± m
» » cells, interphase:	5	10	20	19	19	14	10	1	2	100	$3{,}36 \pm 0{,}17$
» » » II—M ....:	5	7	19	28	19	14	6	2		100	$3{,}25 \pm 0{,}15$

From a comparison between these average values and the average number of dividing univalents, $2{,}59 \pm 0{,}11$, it is evident that more than half of the split univalents must be eliminated. This inference is borne out further by the fact that many of the eliminated bodies are obviously undivided.

The degree of elimination was further studied by chromosome counts at second metaphase. The chromosome number in 125 II—M plates was found to vary from 7 to 13 and was on the average $9{,}58 \pm 0{,}13$. The distribution was as follows:

Number of chromosomes	7	8	9	10	11	12	13
» » plates	5	25	38	20	25	10	2

In 20 cells both plates could be counted. In seven cells the sum of the chromosomes of both plates was higher than 21, the distributions being the following: 12—12 (1 cell), 11—12 (2 cells), 11—13 (1 cell), 9—13 (1 cell), 10—12 (1 cell) and 10—15 (1 cell). In these cases the halves of the dividing univalents have at least in part been



Figs. 44—53. Meiosis in triploid *Dactylis* hybrids (continued). Figs. 44—45, first telophase with eliminated chromosomes; Fig. 46, interphase with micronuclei; Figs. 47—49, II—M, polar view; Fig. 47, 9 and 11 chromosomes respectively in the plates, two eliminated chromosomes at a different level; Fig. 48, 11 + 13 chromosomes and two eliminated bodies; Fig. 49, 8 + 11 chromosomes in the plates, 5 chromosomes eliminated; Fig. 50, II—M, side view, several chromosomes outside the metaphase groups; Fig. 51, II—A with lagging chromosomes; Fig. 52, II—A, polar view, separately drawn; distribution 9—2—9; Fig. 53, tetrad with micronuclei. — Magnification: Figs. 47—49 and Fig. 52, 3375 ×, the other figures 2250 ×.

included in the interphase nuclei. Such half univalents could in some cases be recognized by their smaller size. In Fig. 48, for instance, there are 11 + 13 chromosomes and two small eliminated bodies. Of the 24 chromosomes in the plants 8 must be the halves of four divided univalents. The distribution at I—A in this case was probably 7—5—9, but the halves of one univalent have been eliminated. — In the remaining 13 cells the total chromosome number in the plates was equal to 21 or generally lower, and eliminated bodies were frequent. The following distributions were found (the frequency in brackets): 9—11 (6), 9—12 (2), 9—10 (2), 10—11 (1), 8—11 (1) and 8—10 (1). — In Fig. 47 there are 9 + 11 chromosomes in the plates and two eliminated bodies, which evidently correspond to a divided univalent; in Fig. 49 there are five eliminated bodies and 8 + 11 chromosomes in the plates. In the plate to the right one chromosome is conspicuously small and no doubt represents half a univalent. In this case the anaphase distribution was probably 8—3—10. Five of the half univalents have been eliminated and only one half has reached one of the interphase nuclei.

D. SECOND ANAPHASE AND TELOPHASE (FIGS. 51—53).

As a large number of half univalents are eliminated it is to be expected that the number of lagging chromosomes at II—A will be relatively low and lower than 2,59, the number of univalents dividing at I—A. This was, indeed, found to be the case. Three slides (from the same fixation) were studied and found to have the following numbers of lagging chromosomes at II—A:

Number of lagging chromosomes:	0	1	2	3	4	5	n	M
» » cells of slide 1 .....:	16	17	25	17	6	3	84	1,87
» » » » » 2 .....:	25	22	14	13	3	1	78	1,36
» » » » » 3 .....:	5	7	22	15	4	2	55	2,22
Total:	46	46	61	45	13	6	217	1,77 ± 0,09

The number of lagging chromosomes varies somewhat in different slides, but the average number 1,77 ± 0,09 may be taken as characteristic of the triploid hybrid under discussion. Eliminated chromosomes are frequently seen also at this stage but they are easy to distinguish from the lagging chromosomes. The latter are elongated and somewhat similar to somatic chromosomes (Fig. 51).

The number of chromosomes could be counted in 33 II—A plates. The values obtained were distributed in the following manner:

Number of chromosomes .....:	7	8	9	10	11
» » II—A plates .....:	2	10	13	5	3

The average value is 8,91 ± 0,18. Assuming that all the chromosomes lagging at II—A are included in the daughter nuclei, the average chromosome number of the (male) gametes would be 9,80 (8,91 + half of 1,77) instead of 10,50 if there was no elimination of chromosomes. It is probable, however, that some chromosomes are also eliminated at the second division, since at the second telophase (Fig. 53) the total number of eliminated bodies (in the entire pollen mother cell) was found to be somewhat higher than the number of bodies outside the interphase nuclei. The former value was found to be 3,76 ± 0,18, the latter (as already recorded) 3,36 ± 0,17.

Though it is difficult to estimate accurately the degree of elimination, it is evident from the preceding report that such elimination is rather frequent and that

the average chromosome number in the gametes is lower than half of the somatic number. — Though meiosis is rather irregular, most of the pollen mother cells at the tetrad stage were found to be divided into four cells, only 6 pentads being observed among 100 p. m. c. examined.

4. CHROMOSOME VARIATION IN THE PROGENY OF GLOMERATA × ASCHERSONIANA HYBRIDS.

As already mentioned, the triploid *glomerata* × *Aschersoniana* hybrids were male sterile, but progeny could be obtained by back crosses with both parent species. These crosses were in part performed in isolation cages in the greenhouse, in part in the field. In both cases single triploid specimens were placed in the midst of plants belonging to a certain biotype of *glomerata* or *Aschersoniana*. In the field a number of clones, belonging to the Forage Crop Department of Svalöf, were utilized. As these clones were separated from other clones, and as the triploid hybrids were surrounded on all sides by a large number of clone plants with the same genotypical constitution, the chances of intermixture with foreign pollen were very slight. The seeds produced by the triploid mother plants showed all transitions from very poor and almost empty seeds, uncapable of germination, to seeds of quite normal appearance. The crosses triploid × *glomerata* gave a better result than crosses with the other parent species, but also in this combination a small progeny was obtained.

A. TRIPLOID × ASCHERSONIANA.

In 1933 two different triploids, pollinated by *Aschersoniana* (in the field), gave some seeds. In the following spring thirty seeds of this cross were put to germination on moist blotting paper and 16 plants were obtained. The chromosome numbers of these plants were determined with the following result:

	14	15	16	17	18	19	20
Chromosome number .....:							
Progeny of hybrid No. 1 (32—113):	1	2	1	3	1	—	3
» » » » 2 (32—109):				1	1	1	2
Total:	1	2	1	4	2	1	5

In spite of the low number of individuals all chromosome numbers between 14 and 20 are represented. The plants are too few in number to allow any conclusions as to the possible occurrence of selective processes in this case, but if such processes occur they cannot be very pronounced.

B. TRIPLOID × GLOMERATA.

For the purpose of producing polyploid *Dactylis* types with higher chromosome numbers than *glomerata*, progeny was raised several times from triploid hybrids pollinated by *glomerata*. Though no formation of unreduced gametes had been observed at meiosis on the male side, there was still a possibility of the *Dactylis* hybrids, like most other triploids, forming a low frequency of unreduced ovules. Chromosome counts in the progenies obtained proved this assumption to be correct. Besides a majority of individuals, resulting from the union of reduced female and male gametes, *a considerable proportion of the plants proved to be pentaploid or approximately pentaploid* (Table 4). These plants, which have about 35 chromosomes, must be the result of a union between unreduced ovules and ordinary reduced *glomerata* gametes.

In the main the chromosome numbers of the 238 plants examined vary between 22 and 41, all numbers between these values being represented with the exception of 23 and 39. The distribution of these chromosome numbers is obviously bimodal, the lower maximum being represented by plants with 27 chromosomes, the higher maximum by plants having 2n = 35. Although the two groups cannot be sharply defined,

TABLE 4. *Chromosome numbers in the back cross triploid × glomerata.*

Field numbers	Somatic chromosome number: 22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	... 56	n	Percentage of plants with 32—56 chromosomes
32—103 × Skandia II ...			1	3	1	—	—	—	—	1	3	5	1									15	66,7 ± 10,5
32—104 × » ...			1	—	2	—	—	—	—	—	1	—	1	1	—	—	—					7	57,1
32—107 × » ...			2	—	—	1	—	—	—	—	—	—	1	5								9	66,7
32—111 A × » ...		3	—	2	3	13	27	22	10	3	3	1	6	4	8	1	1	—	2	1		111	22,5 ± 3,9
32—112 × » ...					2	5	4	2														13	0,0
32 × » ...					1	5	9	4	1	—	—	1	1	—	1	—	—	—	—	—	1	24	16,7 ± 8,3
»Triploid» × » ...					8	17	20	9														54	0,0 ± 5,5
33 × »1098»					1	2	—	2														5	0,0
Total	3	—	2	18	43	65	41	11	3	3	2	9	7	16	8	1	1	—	3	1	—	1	238	20,7 ± 2,6

it seems appropriate to draw the boundary-line between the chromosome numbers 31 and 32. By means of this boundary-line the 238 plants examined will be divided into one main group with chromosome numbers ranging from 22 to 31 and one minor group with chromosome numbers ranging from 32 to 56. In the latter group there are 49 individuals, corresponding to $20{,}7 \pm 2{,}6$ per cent of the total plant number. Thus not less than one fifth of the plants must have arisen from unreduced or approximately unreduced gametes.

As is evident from Table 4, the percentage of approximately pentaploid plants is quite different in different progenies and ranges from 0,0 to 66,7 per cent. Some of these differences are significant. Comparing, for instance, the average values $66{,}7 \pm 10{,}5$, $22{,}5 \pm 3{,}9$ and $0{,}0 \pm 5{,}5$ the following differences will be obtained [1]: $(66{,}7 \pm 10{,}5) - (0{,}0 \pm 5{,}5) = 66{,}7 \pm 11{,}9$ (D/m = 5,6); $(66{,}7 \pm 12{,}2) - (22{,}5 \pm 3{,}9) = 44{,}2 \pm 12{,}3$ (D/m = 3,5) and $(22{,}5 \pm 3{,}9) - (0{,}0 \pm 5{,}5) = 22{,}5 \pm 6{,}7$ (D/m = 3,4). From these figures it is evident that at least three of the progenies studied differ with regard to the proportion of pentaploids. The cause of these differences has not been studied but it most probably consists of genotypical differences between the mother plants, leading to different proportions of unreduced ovules. Since no formation of unreduced gametes was observed at meiosis in the p. m. c., the high percentage of pentaploids suggests that the mode of meiosis may be quite different on the male and female side, just as in the *Pygaera* hybrids studied by FEDERLEY (1931). It is possible, however, that the percentage of unreduced ovules is rather low, but that such ovules and embryo-sacs have better viability than most other female gametophytes. It is indeed quite probable that of the gametes formed those with the euploid numbers 7, 14 and 21 are more functional than the aneuploid gametes (at least more functional than gametes with numbers intermediate between 7 and 14). Thus, though the euploid gametes must be quite rare in comparison with aneuploid gametes, it is probable that the former play a relatively greater rôle in the production of progeny. This may account for the rather high percentage of pentaploid plants.

It is evident that also zygotic selection is at work. In the main group of plants, with chromosome numbers ranging from 21 to 31, there is a striking excess of plants with 26, 27 and 28 chromosomes. In the absence of any selection the average chromosome number in this group should be $^{21}/_{2} + 14 = 24{,}5$. However, on account of chromosome

[1] The standard errors were calculated on basis of the total average distribution (20,7 : 79,3).

elimination at meiosis in the triploids the average chromosome number of the gametes must be lower and was estimated to have the maximum value 9,80. Thus, in the absence of any competition the group of plants under discussion should not have an average chromosome number exceeding 23,80. In reality the average value was found to be 26,8 and *of the 189 plants involved all but three had a higher chromosome number than the expected value 23,8.* This might be assumed to be due exclusively to gametic selection and a preference of female gametes with 14 or approximately 14 chromosomes. However, if only gametic selection were involved, there should be a group of plants of the same or even greater size (on account of chromosome elimination at meiosis), corresponding to ovules with 7 or approximately 7 chromosomes. Such plants should have $2n = 21$ or slightly higher numbers. *This group is almost entirely lacking* (Table 4) and there are only three plants having $2n = 22$.

I can only interpret this result along the same lines as similar cases previously discussed (MÜNTZING, 1933 a), *viz.* that such zygotes as result from the union of gametes with approximately the same chromosome number have better viability than zygotes resulting from the union of gametes with rather different chromosome numbers. In the latter case the development of the zygotes is arrested by purely quantitative changes. Consequently, *in the present case the union of gametes with 14 and 7* (or approximately 7) *chromosomes will lead to zygotes with poor viability in contrast to those zygotes which result from the union of male glomerata gametes with 14 and female gametes having 14 chromosomes or approximately that number.* As the ratio 14 : 7 is greater than the ratio 21 : 14 it is comprehensible that pentaploid zygotes have better viability and that pentaploid plants may arise in a considerable frequency. Not even the triploid zygotes are quite unviable, for triploid hybrids are known, and a few plants with approximately 21 chromosomes were actually produced from the cross triploid × *glomerata.*

Among the plants with high chromosome numbers there was also an individual which clearly had $2n = 56$. Seven somatic plates of this plant were counted and 56 chromosomes were found in six cases and 55 chromosomes in one case. This plant must have arisen from the exceptional union of a female gamete with 42 (the double somatic number) and an ordinary *glomerata* gamete with 14 chromosomes. Although it is surprising that the resulting zygote proved to be viable it is difficult to imagine any other mode of origin. Other cases are already known

in which gametes with twice the somatic number have been found to be functional, *e. g.* NORDENSKIÖLD (1937), F. NILSSON (*Festuca* hybrids, unpublished results).

Though the maximum class in the pentaploid group has exactly 35 chromosomes, a still greater number of plants have chromosome numbers which are either a little lower or a little higher (Table 4). Fourteen plants have chromosome numbers varying between 36 and 41, and in 18 plants the chromosome numbers range from 32 to 34. — Since the values in Table 4 are »total» values, including a minority of ± values in addition to those accurately determined, the deviations from 35 might possibly be caused by inaccurate chromosome counts. This is not so, however, firstly because the deviations are much too great to be caused by occasional slight errors, secondly because several deviating values were controlled by new counts. The value 41, for example, has been determined by counts of eleven somatic plates, giving the series: 41, 41, 42, 42, 41, 42, 40, 42, 41, 41, 41. That 41 is the correct value was controlled later by meiotic studies (cf. below p. 150). The number 36 has been especially controlled in three plants. The plates examined gave the following three series: Plant No. 1, 36, 36, 36, 36—37, 36; plant No. 2, 36, 36, 34—35, 35—36, 36, 36, 36; and plant No. 3, 36, 36, 36, 36, 36, 36. The number 36 was also determined exactly at meiosis in another plant (cf. below p. 148).

Thus the production of plants in this cross with higher chromosome numbers than 35 is beyond doubt. The occurrence of such plants might in part be due to the functioning of male gametes with somewhat deviating chromosome numbers, for it has been shown that male gametes with one extra chromosome may be functional. The frequency of aberrants in Skandia II (the strain used for pollination in the present cross) may be estimated to be about 15 per cent (Table 2). Making the somewhat improbable assumption that the male gametes with deviating chromosome numbers have the same viability as the corresponding ovules, this value would correspond to a maximum of about 4 per cent of male gametes with higher chromosome numbers than 14. It is evident, however, that the deviations from 35 are too frequent and too great to be caused by the male gametes alone. The only alternative conclusion is that *part of the ovules of the triploid hybrids are not only unreduced but exceed the somatic chromosome number by one or several chromosomes.* This class of unreduced gametes, having more than the somatic number, evidently corresponds to a class of similar size, in which the ovules are almost unreduced but lack one or a few

chromosomes. Such ovules must be responsible for most of the plants, having 34, 33 and 32 chromosomes and form a transition to the ordinary reduced gametes.

Since it is known from the meiotic studies on the male side that some univalents are generally present at I—M, and that these univalents generally divide at I—A, it is quite easy to understand that »unreduced» ovules with a few chromosomes more or less than the somatic number may be formed. For instance, if restitution nuclei are formed, the chromosome number in these nuclei may be somewhat increased owing to division of some univalents. If a second division follows, the half univalents will be distributed at random and give rise to dyads with slightly less or slightly more than the somatic number.

Though it has generally been assumed that unreduced gametes have exactly the somatic chromosome number, some cases have already been described which are of the same type as in *Dactylis*. In the *Crepis* hybrid *capillaris* × *aspera*, NAWASCHIN (1927) observed the formation of dyads with 8 chromosomes, though the somatic chromosome number in this case is only seven. In the progeny of such hybrids (after open pollination) an individual was obtained, which in addition to one *capillaris* genome and two *aspera* genomes contained an extra chromosome. This result, combined with the meiotic observations, strongly indicates that in this case the mother plant had formed a female gamete with one chromosome more than the somatic number, this ovule being fertilized by a male gamete carrying the *aspera* genome.

KARPECHENKO (1927) recorded the somatic chromosome numbers of 301 daughter plants of *Raphanus* × *Brassica* hybrids. In addition to a majority of individuals with $2n = 36$ about 5 per cent of the plants had one or two extra chromosomes. The occurrence of these exceptional individuals demonstrates that also in the *Raphanus* × *Brassica* hybrids unreduced gametes with more than the somatic chromosome number may be formed. Similarly KOSTOFF (1933) obtained some plants in the cross *Nicotiana tabacum* × (*N. sylvestris* × *N. Rusbyi*) which had more than 48 chromosomes. These plants, which were sister plants of normal »triplex» hybrids with $2n = 48$, must have arisen from normal *tabacum* gametes with 24 chromosomes and male gametes from the *sylvestris* × *Rusbyi* hybrid, containing one or a few chromosomes more than the somatic number. — PETO (1933), working with *Lolium* × *Festuca* hybrids, made a back cross between a hybrid derivative with 18 chromosomes and one of the parents, *Festuca arundinacea*. The expected chromosome number was 39, but the plant obtained had

$2n = 42$. The author suggests two explanations, either fragmentation or formation of an unreduced gamete with 3 chromosomes more than the somatic number. As PETO suggests, this gamete might be formed through certain of the univalents splitting before the restitution nucleus was formed.

Quite clear cases of the formation of unreduced gametes with extra chromosomes have been reported by KATTERMANN (1934) and DRYGALSKI (1935). KATTERMANN, working with wheat × rye hybrids, found that in addition to exactly unreduced gametes with 28 chromosomes, ovules with 1—3 extra chromosomes (or an extra fragment) were functional. DRYGALSKI, finally, found several hypertetraploid plants in F_2 of the cross *Saxifraga adscendens* × *tridactylites*. As in the *Raphanus* × *Brassica* case most of the F_1 gametes in the *Saxifraga* hybrid are unreduced. Some of these gametes, however, evidently contain a few chromosomes more (or less) than the majority of the unreduced gametes.

In this connection I wish to make a correction concerning the genus *Galeopsis*. In previous publications (MÜNTZING, 1930, 1932) the so-called artificial *Tetrahit* plant (»AT») obtained from crosses between the diploid species *G. speciosa* and *pubescens* (triploid F_2 plant back crossed to *pubescens*) has been reported to have $2n = 32$. On account of a relatively high frequency of trisomic plants with $2n = 33$ in the progeny of the AT plant and owing to similar progenies from controlled trisomic individuals I began to suspect that the correct chromosome number of the AT plant was 33 and not 32. A re-examination of the old and rather poor slides has now convinced me that $2n = 33$ is in fact the correct number. Most probably, then, this case is analogous to those described above, the AT plant having arisen from the union of a hypotriploid female gamete with 25 and a male gamete with 8 chromosomes. This will be further discussed in another paper.

5. MEIOSIS IN A PENTAPLOID DACTYLIS PLANT.

The pentaploid plant chosen for meiotic studies happened to be a hyperpentaploid with 36 instead of 35 chromosomes. There is reason to believe, however, that the mode of meiosis in this individual is of the same general type as in pentaploids, having exactly 35 chromosomes.

Meiosis in the pentaploids is evidently characterized by the presence of a variable number of quinquevalents, quadrivalents, trivalents, bivalents and univalents (Figs. 54—55). As usual the univalents have a tendency to lag and divide and this leads to a rather marked degree

of chromosome elimination. The gametes formed will on an average receive less than half of the somatic chromosomes.

In support of this general statement the following details may be given. With some difficulty 15 complete *first metaphase* groups were analysed and found to represent 14 different combinations. Only two cells correspond to the same formula. The following configurations were observed:

$1_{VI}+4_{V}+1_{IV}+2_{II}+2_{I}$
$1_{VI}+3_{V}+3_{III}+3_{II}$
$1_{VI}+2_{V}+1_{IV}+3_{III}+3_{II}+1_{I}$
$1_{VI}+2_{V}+1_{IV}+2_{III}+4_{II}+2_{I}$
$1_{VI}+1_{V}+2_{IV}+3_{III}+3_{II}+2_{I}$
$3_{V}+2_{IV}+2_{III}+3_{II}+1_{I}$
$3_{V}+1_{IV}+3_{III}+3_{II}+2_{I}$ (2 cells)
$3_{V}+3_{III}+5_{II}+2_{I}$
$2_{V}+3_{IV}+3_{III}+1_{II}+3_{I}$
$2_{V}+2_{IV}+4_{III}+2_{II}+2_{I}$
$2_{V}+2_{IV}+2_{III}+5_{II}+2_{I}$
$2_{V}+1_{IV}+3_{III}+6_{II}+1_{I}$
$2_{V}+1_{IV}+2_{III}+7_{II}+2_{I}$
$1_{V}+3_{IV}+3_{III}+4_{II}+2_{I}$

The average number of quinquevalents (and hexivalents) per cell is 2,67. The corresponding average values for the other configurations are 1,40 (quadrivalents), 2,60 (trivalents), 3,60 (bivalents) and 1,73 (univalents).

As might be expected the extra chromosome present sometimes causes the formation of a hexivalent (Fig. 54, to the left) but is usually a member of a smaller association. *The number of quinquevalents (2,67) is lower than the average number of quadrivalents in the tetraploids (3,45), just as this value is lower than the average number of trivalents (4,58) in the triploids.* This decrease is in accordance with the chiasma theory of chromosome pairing (cf. DARLINGTON, 1937).

About half of the quinquevalents (and hexivalents) were strictly zig-zag arranged, adjacent chromosomes going to opposite poles. Typical W-shaped quinquevalents of this kind are, for instance, represented in Fig. 54, 4th configuration from the right and in Fig. 55, 3rd configuration from the left. The quadrivalents observed were generally present as zig-zag chains or as other zig-zag configurations. Only 3 quadrivalents of 21 were not arranged in zig-zag.

At first anaphase most of the cells contained one or a few dividing univalents. In 109 cells examined (from 2 slides) the following frequency was observed:

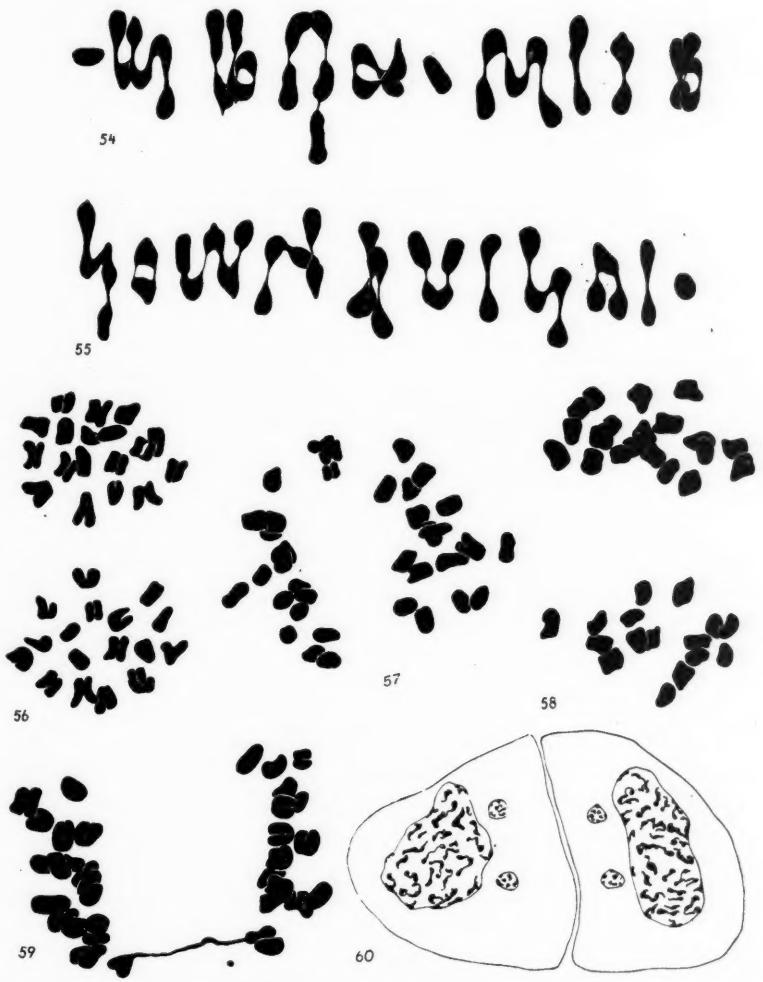
Number of dividing univalents ....:	0	1	2	3	4
» » cells .....:	15	35	36	20	3

The average number is $1{,}64 \pm 0{,}10$. Since the average number of univalents at I—M was found to be 1,73 it is evident that most or all univalents divide at I—A.

In some cells at this stage the total chromosome number could be determined. The following rather variable distributions were found (the number of cells in brackets): 17—3—16 (3 cells, Fig. 57), 17—19 (3), 17—1—18 (2), 17—2—17 (2), 16—20 (2 cells, Fig. 58), 18—18 (1 cell, Fig. 56), 16—2—18 (1), 19—1—16 (1) and 15—4—17 (1). — In one cell (Fig. 59) showing the distribution 17—19, the simultaneous occurrence of a chromatin bridge and a small fragment indicated that crossing-over had occurred in an inverted segment.

As usual in *Dactylis*, when univalents lag and divide at I—A, a fairly high number of eliminated bodies were observed at *interphase*. Fig. 60 shows a cell at this stage with 4 micronuclei, probably corresponding to 2 divided univalents. In two slides examined the total number of micronuclei was as follows:

Number of micronuclei .....:	0	1	2	3	4	5	6	7
» » cells .....:	26	7	29	13	18	5	3	1



Figs. 54—60. Meiosis in pentaploid *Dactylis* ($2n = 36$). Figs. 54—55, I—M, side view (separately drawn); Fig. 54, $1_{VI} + 4_V + 1_{IV} + 2_{II} + 2_I$; Fig. 55; $3_V + 2_{IV} + 2_{III} + 3_{II} + 1_I$; Figs. 56—59, I—A; Fig. 56, distribution 18—18; Fig. 57, distribution 16—3—17; Fig. 58, distribution 16—20; Fig. 59, chromatin bridge and small fragment, distribution 17—19; Fig. 60, interphase with micronuclei. — Magnification: Fig. 60, 1688 ×, the other figures, 3375 ×.

The average number is $2{,}22 \pm 0{,}17$ and consequently less than the double number of dividing univalents ($1{,}64 \pm 0{,}10$). This indicates that some of the split univalents are included in the interphase nuclei. It is also probable that some univalents are eliminated before division is completed, just as in the triploid hybrids. This is evident from Fig. 61, showing three eliminated bodies outside the II—M plates. Judging from both appearance and chromosome number ($17 + 17$ in the plates) the large eliminated body represents an undivided eliminated univalent, the two small bodies a split univalent.

The number of chromosomes in the *second metaphase* plates was found to have the average value of 17,10 and varied from 15 to 21 as shown by the following distribution:

Number of chromosomes ...:	15	16	17	18	19	20	21
» » cells .....:	9	16	23	12	5	5	1

The chromosome number could also be counted in 28 *second anaphase* groups and was found to vary between 15 and 20, the average number in this case being 17,25. Fig. 62 shows a cell in which all four II—A groups could be counted. In this case the distribution at I—A was 16—20. — A low frequency of lagging chromosomes was observed at II—A, but it was found difficult to distinguish these chromosomes with certainty from chromosomes eliminated in the first division.

Finally, it should be mentioned that the number of micronuclei in the young tetrads was found to vary between 0 and 8, the average of 112 cells being 1,79. The distribution was of the same type as at interphase.

6. NOTE ON THE MEIOSIS IN A PLANT WITH 41 CHROMOSOMES.

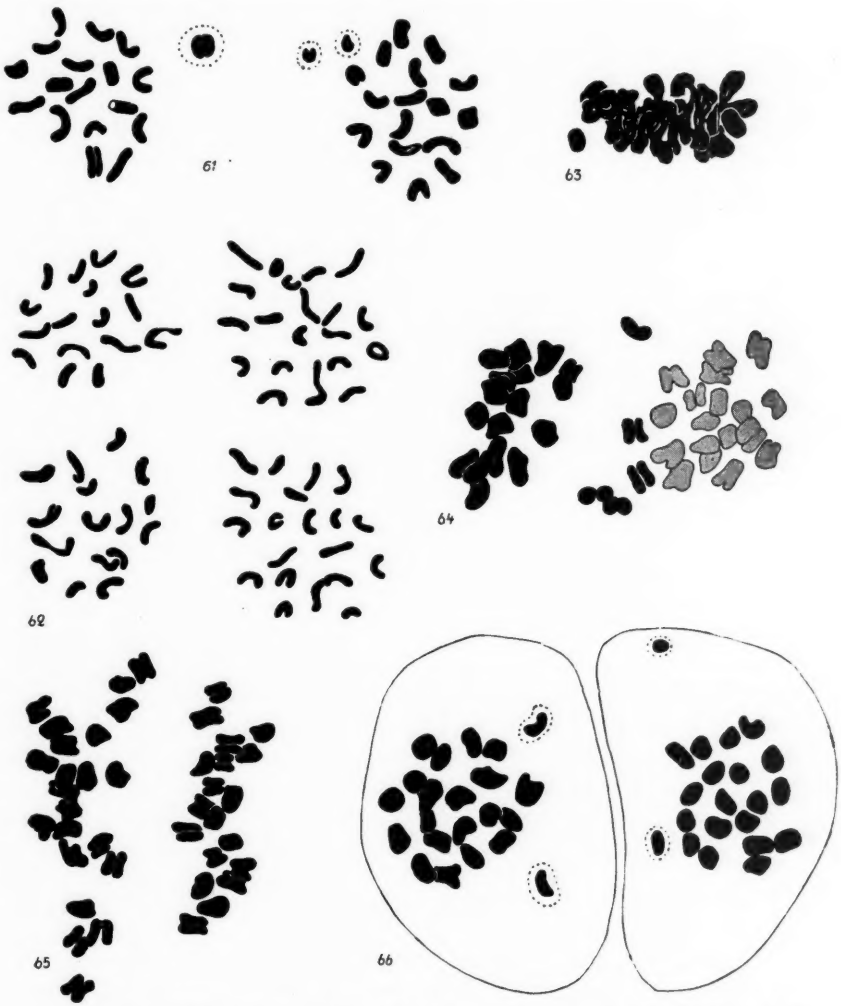
With the exception of 56, the highest chromosome number directly obtained from the cross triploid $\times$ *glomerata* is 41. The carrier of this number is a fairly vigorous plant compared with the plant with 56 chromosomes, which died before flowering.

With the aid of some aceto-carmine preparations some preliminary meiotic observations have been made in the 41-chromosome individual. Generally speaking these observations show that the plant in question is cytologically unstable, though the irregularities are not very great. A low number of univalents occur and cause a certain amount of chromosome elimination.

The somatic chromosome number was found to be correctly determined as is evident from Fig. 65, representing a first anaphase distribution with $19 + 22$ chromosomes. Another cell at the same stage showed the same distribution and in another two cells the numbers 20—21 and 17—5—19 (Fig. 64) were counted. At I—A from 0 to 6 univalents were observed to divide. The following frequency was found:

Number of dividing univalents ....:	0	1	2	3	4	5	6
» » cells .....:	14	20	20	13	7	1	2

The average number is 1,87, about the same number as in the 36-chromosome plant studied (1,64).



Figs. 61—62, meiosis in pentaploid *Dactylis* (continued); Fig. 61, II—M, 17 + 17 chromosomes in the plates, three bodies eliminated; Fig. 62, II—A, polar view, separately drawn, distribution $\frac{16—16}{20—20}$. — Figs. 63—66, meiosis in a *Dactylis* plant with 41 chromosomes. — Fig. 63, I—M, side view, two univalents visible; Fig. 64, I—A, distribution 17—5—19; Fig. 65, I—A (flattened by pressure), distribution 19—22; Fig. 66, II—M, 19 + 20 chromosomes in the plates, 4 chromosomes eliminated. — Magnification: Figs. 61—62, 3375 ×, Figs. 63—66, 2530 ×.

A low number of univalents was also observed outside the first metaphase groups (Fig. 63) but in the aceto-carmine preparations available no exact counts could be made. It was also impossible to analyse the I—M configurations.

At interphase the number of eliminated bodies varied between 0 and 8 and was found on the average to be 1,96. This value closely corresponds to the average number of bodies (1,98) found to be situated outside the second metaphase plates (cf. Fig. 66). The latter value was determined by counts of 81 cells. At II—T and the young tetrads, finally, the average number of micronuclei was found to be 2,23 (52 cells examined).

These values show that the degree of irregularity and chromosome variation is of about the same order in the present plant as in the 36-chromosome individual studied. The gametes will have variable chromosome numbers, which are on the average lower than half of the somatic number. This view is substantiated by some counts at second metaphase. A total of 21 metaphase plates were examined and found to contain from 18 to 22 chromosomes. The average value was 19,90. The mean value in the gametes will probably be still lower, since in some cases the II—M plates will contain half univalents which lag at II—A. In one cell (Fig. 66) both plates could be counted and were found to contain 19 and 20 chromosomes, two divided univalents being eliminated.

7. CHROMOSOME NUMBERS IN PROGENIES OF PENTAPLOID DACTYLIS TYPES.

In contrast to their triploid mother plants the pentaploid derivatives, obtained from the back cross triploid $\times$ *glomerata*, have quite good fertility on the female as well as on the male side (cf. below p. 184). Isolated pentaploids give plenty of seeds, and progeny after self-fertilisation can thus be raised without difficulty. Hitherto the chromosome numbers of 204 such daughter plants have been determined. The results of these counts are summarized in Table 5. When studying this table, two facts are at once obvious: 1) *The chromosome numbers are quite variable and range from 28 to 41*, 2) *The average chromosome numbers of the eight progenies are all lower than the chromosome numbers of the respective mother plants*, the differences ranging from 1,00 to 2,62. These results might have been predicted from the meiotic observations, which demonstrated the formation of gametes with variable chromosome numbers and the occurrence of chromosome elimination. The plant with $2n = 36$, in which meiosis was studied, is the mother plant of the progeny 35—4. The average chromosome number of the (male) gametes in the mother plant was estimated at about 17,2, the average chromosome number of the progeny was 33,50 (Table 5). The agreement between these data is rather good. The same average decrease of the chromosome number in all the other progenies strongly indicates that meiosis in the mother plants is of the

same main type in all cases, though there seem to be slight differences in the degree of chromosome elimination.

The progenies from pentaploids were raised with the purpose of

TABLE 5. *Chromosome numbers in progenies of pentaploids.*

Field number	Somatic chromosome number: 28	29	30	31	32	33	34	35	36	37	38	39	40	41	n	M	Chromosome number of the mother plant	Difference
35—1	1	2	5	5	9	2	—	—	3						27	31,59	± 34	— 2,41
35—5				1	5	4	7	7	1	—	2				27	34,00	± 35	— 1,00
35—04				2	1	2	—	—	—	1	—	—	—	1	7	34,00	35	— 1,00
35—05		1	6	3	2	7	1	1	1	1	1				24	32,38	35	— 2,62
35—2					2	3	2	10	2	2	1	1			23	34,96	36	— 1,04
35—3					1	1	3	7	2	—	1				15	34,80	36	— 1,20
35—4				1	3	6	4	3	—	1					18	33,50	36	— 2,50
34—33	1	1	1	3	10	7	14	14	6	1	4	1			63	33,98	36	— 2,02

obtaining plants with higher chromosome numbers, especially such as have 42 chromosomes. Though this result was not obtained in the first generation, such plants will no doubt be produced by further breeding from the low proportion of plants having 37 to 41 chromosomes. Three daughter plants have already been raised from the 41-chromosome plant, obtained directly in the cross triploid × *glomerata* (Table 4). These daughter plants had the numbers ± 40, ± 41 and 48, a result which is in accordance with the irregular meiosis observed in the mother individual (cf. above p. 150). As this plant has rather good fertility (at least on the male side) it will be easy to raise new and larger progenies. Most individuals in these progenies will certainly be rather unbalanced but, theoretically, it should be possible to obtain relatively stable types with seven sets of six chromosomes instead of seven sets of four chromosomes as in ordinary *D. glomerata.*

8. CHIASMA FREQUENCIES IN TRIPLOID, TETRAPLOID AND PENTAPLOID DACTYLIS.

In the same way as in *Dactylis Aschersoniana* (Table 1) the chiasma frequencies have been counted in the other *Dactylis* types studied. The data obtained are summarized in Table 6. Typical *glomerata* with 28 chromosomes has evidently the same average chiasma frequency per chromosome as the *glomerata* aberrants with $2n = 27$ and $2n = 29$, the values obtained being $0{,}823 \pm 0{,}016$, $0{,}802 \pm 0{,}017$ and $0{,}817 \pm 0{,}015$ re-

spectively. In striking contrast to these values *both the pentaploid and especially the triploid were found to have much lower chiasma frequencies,* the average values being $0{,}742 \pm 0{,}017$ and $0{,}690 \pm 0{,}007$ respectively. Both values are significantly lower than those of *glomerata.* On first thoughts this decrease might be considered to be due to the fact that the triploid hybrids are species hybrids and the pentaploids derivatives from these species hybrids. In other words, the lower chiasma frequency might be due to incomplete homology between the *Aschersoniana* and *glomerata* genomes. That this cannot be a correct explanation, however, is evident when the following facts are considered.

In the triploid hybrid the mode of chromosome association at meiosis strongly indicates that the three genomes present are completely homologous. The average number of trivalents was found to be as high as 4,58 and the maximum association, 7_{III}, was observed several times. Thus, the low chiasma frequency per chromosome in the triploid can scarcely be due to incomplete homology. Now, in the other pure species, *D. Aschersoniana,* the chiasma frequency per chromosome was found to be about as high or only slightly lower than in *glomerata,* the four average values obtained being $0{,}776 \pm 0{,}011$, $0{,}777 \pm 0{,}017$, $0{,}784 \pm 0{,}009$ and $0{,}824 \pm 0{,}010$ (Table 1). Consequently, in *Dactylis the types having even multiples of seven (14 and 28) have a higher chiasma frequency per chromosome than the types with odd multiples (21 and 35).*

For pure mechanical reasons it is comprehensible that the chiasma frequency per chromosome must be lower in the triploid

TABLE 6. *Chiasma frequencies in triploid, tetraploid and pentaploid Dactylis.*

Type and chromosome number	Total number of chiasmata: 12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	n	M ± m	Chiasma number per chromosome
Triploid *Dactylis*, 2n = 21.....	1	4	25	11	7	2													50	$14{,}50 \pm 0{,}15$	$0{,}690 \pm 0{,}007$
D. glomerata, »pure», 2n = 28...							2	—	1	3	3	3	7	3	2	1			25	$23{,}04 \pm 0{,}46$	$0{,}823 \pm 0{,}016$
» » aberrant, 2n = 27						1	—	1	2	2	2	10	1						20	$21{,}65 \pm 0{,}47$	$0{,}802 \pm 0{,}017$
» » 2n = 29							1	—	3	1	2	2	6	4	4	1	1		25	$23{,}68 \pm 0{,}49$	$0{,}817 \pm 0{,}015$
»Pentaploid» *Dactylis*, 2n = 36...													2	1	5	1	1	4	14	$26{,}71 \pm 0{,}49$	$0{,}742 \pm 0{,}017$

than in the diploid and lower in the pentaploid than in the tetraploid. As pointed out by MATHER (1935) the maximum length which can be paired at pachytene, in a triploid with entirely homologous chromosomes is not greater than in the diploid, since in triplods only two chromosomes come together at any one point during prophase. From that point of view the total chiasma number should not be higher in the triploid than in the diploid, and therefore the chiasma number per chromosome should be much lower in the triploid than in the diploid. For the same reasons the pentaploid must have a lower chiasma number per chromosome than the tetraploid.

In the present case, however, this explanation cannot cover the whole truth, since the total chiasma frequency was actually found to be much higher in the triploid than in the diploid and also significantly higher in the pentaploid than in the tetraploid (Table 6). In *Tradescantia bracteata*, ANDERSON and SAX (1936) also found a greater total chiasma frequency in an autotriploid form than in the corresponding diploid. Just as in *Dactylis* the chiasma number per chromosome was somewhat lower in the triploid than in the diploid, the average values obtained being 0,8 and 1,0 respectively.

From data to be given below it is evident, however, that the lower chiasma frequency in triploids and pentaploids is at least in part connected with the fact that *trivalents and quinquevalents, on account of their odd chromosome number, are not capable of forming closed rings.* As is well known the free ends of these configurations are not homologous, in contradistinction to the free ends of chain quadrivalents. Hence, the average chiasma frequency per chromosome in trivalents and quinquevalents will be lower than in the quadrivalents, which are often ring-shaped.

In the different types of multivalents observed the following chiasma frequencies were actually obtained. Of 225 trivalents in the triploid hybrid 183 had two and 42 three chiasmata. The average chiasma number is consequently $2{,}19 \pm 0{,}03$ per trivalent and $0{,}73 \pm 0{,}01$ per chromosome. In »pure» *glomerata* ($2n = 28$) 30 quadrivalents had three chiasmata and 53 had four, the average value being $3{,}64 \pm 0{,}05$ per quadrivalent and $0{,}91 \pm 0{,}01$ per chromosome. The difference $0{,}91 - 0{,}73 = 0{,}18$ is great and significant.

In the *glomerata* aberrants exactly the same high chiasma frequency was found to be characteristic of the quadrivalents. In the type with 27 chromosomes 23 quadrivalents had three and 40 quadrivalents four chiasmata. This corresponds to 3,63 chiasmata per quadrivalent

and $0{,}91$ per chromosome, just the same value as before. In the pentasomic type, finally, the corresponding value was found to be $0{,}92$ (3,66 per quadrivalent, 25 quadrivalents with three, 49 with four chiasmata). This very clear difference in chiasma number per chromosome in the trivalents and quadrivalents is enough to account for the difference in the total chiasma frequency between triploids and tetraploids. If the average chiasma frequency per chromosome was the same in the trivalents as in the quadrivalents it would mean an increase of the total chiasma frequency in the triploids equal to $\frac{(0{,}91 - 0{,}73) \cdot 4{,}58}{7} = 0{,}118$ ($4{,}58$ = the average number of trivalents in the triploid). The chiasma number per chromosome in the triploid was found to be $0{,}690$. Adding $0{,}690$ and $0{,}118$ the value $0{,}808$ will be obtained. The close correspondance between this value and those actually found in *D. glomerata* ($0{,}823$, $0{,}802$ and $0{,}817$) may be taken as evidence that the explanation given is in the main correct.

In the pentaploid 34 quinquevalents were found to have four chiasmata and 5 to have five chiasmata. This gives an average of $4{,}13 \pm 0{,}05$ chiasmata per configuration and $0{,}83 \pm 0{,}01$ per chromosome. The latter value is significantly lower than the corresponding value for the quadrivalent chromosomes in the tetraploid ($0{,}91 \pm 0{,}01$). Hence, the lower total chiasma frequency in the pentaploid may be partly due to the relatively low chiasma number per chromosome in the quinquevalents but it must also be partly due to the presence of trivalents, which are not infrequent in the pentaploid (2,60 per cell). These trivalents were found to be of the same type as in the triploid hybrid.

When counting the chiasmata in the triploid the observation was made that the bivalents in this type were as a rule ring-shaped with two chiasmata, rod-shaped bivalents with only one chiasma being much less frequent than in the other *Dactylis* types examined. And indeed, actual counts revealed a significant and striking difference in this respect between the triploid and tetraploid. In the triploid 107 bivalents had two chiasmata and only 16 had one chiasma. Thus the average value in the triploid is $1{,}87 \pm 0{,}03$ per bivalent and $0{,}94 \pm 0{,}02$ per chromosome. In the tetraploid ($2n = 28$) 82 bivalents had two chiasmata and 84 one chiasma. This gives the corresponding values $1{,}49 \pm 0{,}04$ per bivalent and $0{,}75 \pm 0{,}02$ per chromosome. Thus, *in the triploid the chiasma number per chromosome in the bivalents is 25 per cent higher than in the tetraploid.* Now, as demonstrated above, the average chiasma number per chromosome in the trivalents (in the triploid) was $0{,}73 \pm 0{,}01$ while the corresponding value for the quadrivalent chromosomes in the tetraploid

was significantly *higher* ($0{,}91 \pm 0{,}01$). *In the triploid as well as in the tetraploid there is consequently a negative correlation between the chiasma frequencies of those chromosomes present as bivalents and those present as multivalents.* But while in the tetraploid the quadrivalent chromosomes represent the high chiasma number, it is just the opposite in the triploids, the bivalents in this type having a much higher chiasma frequency (per chromosome) than the trivalents. This can scarcely be interpreted in any other way than that there is a rather marked tendency in the cells to keep the total chiasma number constant. — As mentioned above (p. 121) the same tendency was met with in *D. Aschersoniana*. — As the trivalents are incapable of forming closed rings, their chiasma number will be low, but the cells »try» to compensate this decrease by a very marked increase in the chiasma number of the bivalents. However, this effort to a regulation back to the total chiasma frequency, characteristic of the genus, is only partially successful (probably on account of the low average number of bivalents) and the triploid has therefore a significantly lower chiasma frequency per chromosome than the tetraploids and diploids.

Though not universal the occurrence of such a negative correlation of chiasma frequencies is evidently rather wide-spread and has recently been found both in animals and in plants. The real nature and causes of this phenomenon are not known, but a certain average chiasma frequency per cell seems to be characteristic of each species or genus just as other physiological and morphological characters. This has interesting genetical consequences, *viz.* that the degree of crossing over in one chromosome pair is not independent of the degree of crossing over in other pairs. Genetical data of this kind have already been obtained in *Drosophila* (MORGAN, BRIDGES and SCHULTZ, 1933). The chiasma number per cell has a more or less marked tendency to remain constant but is evidently influenced by environmental as well as genetical factors. In ordinary rye, for instance, MATHER and LAMM (1935) found in general the intra-cell variance to be greater than the inter-cell variance, but in inbred rye LAMM (1936) found no negative correlation. In a recent paper MATHER (1936) has reported a series of new results and gives a review of the whole question. Further discussion is therefore superfluous in the present paper. It should only be mentioned that in triploid *Tulipa*, MATHER (l. c.) found a very marked negative correlation. Judging from the present results in *Dactylis* this is true also of other triploids.

III. CHROMOSOME NUMBER AND VIGOUR.

1. METHODS.

As the *Dactylis* material studied represents a polyploid series as well as many aneuploid chromosome numbers, it is very well suited for comparative investigations of plant vigour in relation to chromosome number. A further great advantage is the fact that the *Dactylis* species and their derivatives are all perennial. Therefore, and as the plants have a pronounced capacity of producing new shoots, the green parts can be cut down entirely and be weighed without killing the individuals. In most of the material the plants were cut and weighed twice in the summer, the first time in July, shortly after the flowering, the second time in September (the re-growth). Height measurements have also been undertaken, and in a few cases the vigour was estimated simply by ocular gradation. Though the latter two methods give values which are positively correlated with the true plant vigour, undoubtedly the best method is to weigh the plants.

The ideal way to compare the effects of different chromosome numbers would have been to grow the entire material simultaneously under identical environmental conditions. This was impossible, however, since the progenies were raised in different years and were grown in different fields. In order to combine the data from different families it was found necessary to calculate the *relative vigour,* i. e. the individual absolute values expressed in percentage of the mean value [1] of the progeny in question. In most of the material the absolute values used represent the total weight of three weighings (September 1935, July and September 1936).

The standard errors have been calculated in the way devised by FISHER (1936), and are in most cases, when several series of the same type are compared, based on the total Σd^2 and the average variance of all the series. By this method the standard errors may also be given for the means of very small series (or even single variates).

2. PURE SPECIES AND F_1-HYBRIDS.

The difference in chromosome number between *Dactylis Aschersoniana* and *glomerata* is correlated with differences in morphology

[1] In order to obtain this value as accurate as possible it was based on the weights of *all* plants in the progeny, even if the chromosome numbers of some plants had not been determined.

and vigour. The morphological details will be considered in a later chapter, the difference in vigour is evident from the following data.

The diploid species, *D. Aschersoniana*, is much smaller and less vigorous than the tetraploid *glomerata*, and the F_1-hybrids are intermediate. Fig. 67 shows typical plants of *Aschersoniana* (*a*), *glomerata* (*c*) and a spontaneous F_1-hybrid (*b*). The typical difference between the two species is again met with in Fig. 72 (*a* and *b*). Though the difference in vigour is a typical species difference and of such a magnitude that special measurements are almost superfluous, the following weight figures may be given (Table 7). In 1935 ten biotypes of *D. Aschersoniana* were weighed at the same time as five different triploid hybrids and three different clones of *D. glomerata*. The number of clone plants in these three categories were 12, 6 and 18 and the average values 175, 467 and 572 respectively.

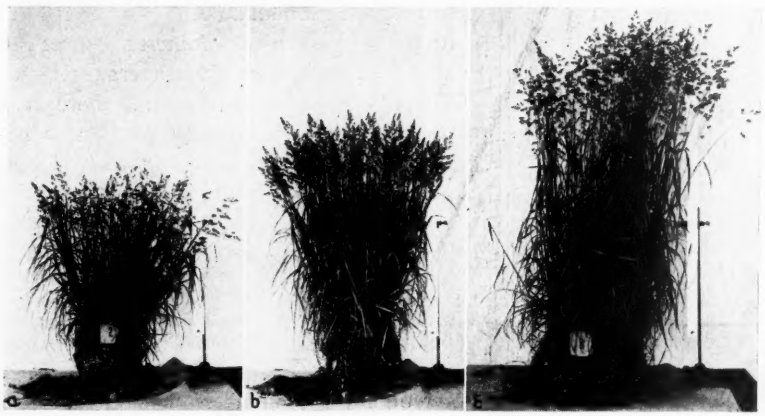


Fig. 67, *a*) *Dactylis Aschersoniana*; *b*) Spontaneous *glomerata* × *Aschersoniana* hybrid; *c*) *Dactylis glomerata*.

In the autumn of 1935 this material and some additional triploids were transplanted to another field, and at the same time the number of clone plants was increased by dividing the plants into two or more pieces. The big *glomerata* plants were divided into more pieces than the *Aschersoniana* plants, the new clone plants of both species originally being of about the same size. Nevertheless, in the following summer (1936) the average weight of the *glomerata* plants was about twice as great as that of *Aschersoniana* (Table 7). The triploids were again intermediate but on account of very hard vegetative division (in order to increase the number of triploid individuals as much as possible) the average value of 1936 was lower than is typical. The values obtained in 1935 illustrate the true situation better.

TABLE 7. *Plant weight in the two Dactylis species and their F_1-hybrids.*

Year	Species and biotypes	Plant weight: 0 100 200 300 400 500 600 700 800 900 1000 gr.	n	M
1935	*D. Aschersoniana* (10 biotypes)	2 8 — 1 1	12	175
»	Spontaneous triploids (5 individuals).....	1 2 — 1 2	6	467
»	*D. glomerata* (3 biotypes)	1 1 3 2 4 1 3 — 3	18	572
1936	*D. Aschersoniana* (10 biotypes)	17 7 5	29	109
»	Spontaneous triploids (11 individuals).....	20 21 1 3 1	46	128
»	*D. glomerata* (3 biotypes).....	5 12 6 5 — 1	29	205

The difference between *Aschersoniana* and *glomerata*, however, is probably somewhat exaggerated in this case, as the *glomerata* biotypes measured were the mother clones of three commercial varieties.

Though several other *glomerata* biotypes (including the wild biotype from Altai) have been observed (cf. Table 2), these were not grown under such conditions as to allow a direct comparison with *Aschersoniana*. All of them were found to be typical *glomerata*, however, also with respect to their average vigour. There can be no doubt that there is a positive and very marked correlation between chromosome number and vigour, which is characteristic of the two species in general.

3. TRIPLOID × ASCHERSONIANA.

Though only a very limited number of plants was obtained from the cross triploid × *Aschersoniana*, the effect of different chromosome numbers in this case was found to be quite pronounced. As mentioned above (p. 141), all chromosome numbers between 14 and 20 were represented. The average vigour of the two progenies was very poor, but there were great differences between the individual plants.

The plants under discussion were raised from seeds germinated in April 1934. The young plants wintered in the field, but in the spring of 1935 their appearance was on the average very poor, and therefore the entire material was transplanted into the green house and carefully grown in pots. At the time of flowering the vigour of the plants was estimated, using a scale from 1 to 8 (8 representing the most vigorous individuals). In the autumn of 1935 the plants were again transplanted into the field. In 1936

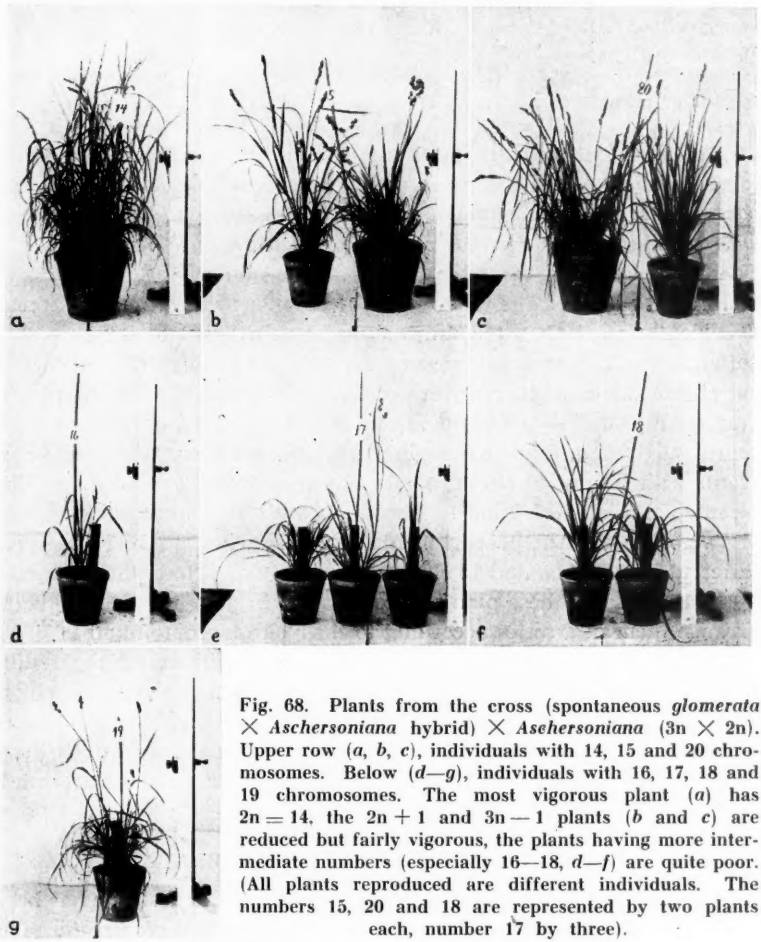


Fig. 68. Plants from the cross (spontaneous *glomerata* × *Aschersoniana* hybrid) × *Asehersoniana* (3n × 2n). Upper row (*a*, *b*, *c*), individuals with 14, 15 and 20 chromosomes. Below (*d*—*g*), individuals with 16, 17, 18 and 19 chromosomes. The most vigorous plant (*a*) has 2n = 14, the 2n + 1 and 3n — 1 plants (*b* and *c*) are reduced but fairly vigorous, the plants having more intermediate numbers (especially 16—18, *d*—*f*) are quite poor. (All plants reproduced are different individuals. The numbers 15, 20 and 18 are represented by two plants each, number 17 by three).

they were cut and weighed twice in the same manner as the other *Dactylis* material.

Of the 16 seedlings originally raised, and cytologically examined, two died at an early stage, the chromosome numbers of these seedlings being 17 and 20. In the winter of 1935—1936 one of the 18-chromosome plants died, consequently 14 plants were measured in 1935 and 13 in 1936. The two sets of data obtained may be summarized in the following table:

Chromosome number .....:	14	15	16	17	18	19	20
Average vigour in 1935 .....:	8,0	5,5	1,0	1,0	1,7	4,0	4,7
Number of plants .....:	1	2	1	3	3	1	3
Average weight in 1936 .....:	475	100	25	42	25	75	125
Number of plants .....:	1	2	1	3	2	1	3

As is evident from the table *the plants with intermediate chromosome numbers* (16, 17, 18) *are very poor, those which approach the parental chromosome numbers are better* (15, 20) *and the only plant with normal vigour has 14 chromosomes, like pure Aschersoniana.* The 1936 values in the table represent the sum of the summer and autumn weighings. The total weight of the plant with 14 chromosomes is evidently more than ten times as great as the weight of the plants with intermediate chromosome numbers. The striking effects of different chromosome numbers in this cross is illustrated best by the photographs (Fig. 68, *a*—*g*). The vigorous plant with 14 chromosomes is represented by *a*, and in the same row the relatively good vigour of plants with 15 and 20 chromosomes is demonstrated (*b* and *c*, two different plants of each kind). The plant with 19 chromosomes (*g*) is less vigorous, but the poorest plants are those with 16 (*d*), 17 (*e*, three different individuals) and 18 chromosomes (*f*, two different individuals). — In 1935, when these plants were growing in pots, it was possible to arrange them in a series according to their chromosome number. This series constituted a beautiful living curve with a minimum in the middle and a maximum at the extreme values. Though the number of individuals in the present cross is rather low the results obtained are certainly significant, and they demonstrate that also in *Dactylis* there is a marked decrease in vigour if the chromosome numbers are intermediate between the 2n and 3n condition.

4. PURE AND ABERRANT D. GLOMERATA.

The correlation between chromosome number and vigour has been studied in all available progenies of pure and aberrant *glomerata*. The results obtained are summarized in Tables 8—9. In these tables only such individuals are included as have their chromosome numbers accurately determined. On the basis of the »total» values (cf. Table 2), correlations of the same type were obtained, but the differences were less distinct.

In the upper part of Table 8 the relative vigour in progenies of pure *glomerata* is given. The material is the same as that given in Table 2, and thus it represents the sum of eight different progenies.

TABLE 8. *Correlation between chromosome number and relative vigour in progenies of pure and aberrant Dactylis glomerata.*

Kind of progeny	Chromosome numbers of the mother plants	Chromosome numbers in the progeny	Relative vigour: 0 20 40 60 80 100 120 140 160 180 200 220 240 260	n	M ± m
Progenies of pure *glomerata*	28	27	1 1 2 2 2 2	10	88,0
		28	3 7 4 10 39 59 10 11 2	145	99,6
		29	1 1 2 2 — 1	7	95,8
		30	1	1	(90,0)
Progenies of *glomerata* aberrants	27, 29, 30	26	1	1	(70,0)
		27	1 — 5 4 4 5 — 2	21	83,4
		28	6 4 16 7 23 7 9 3 4 — 1	80	105,2
		29	3 4 2 17 9 24 3 5 1 1 1 — 1	71	96,4
		30	1 2 3 9 3 4 6 2 1	31	90,6
		31	1 — — — 2 — 1	4	80,0
		32	1 1	2	60,0
Sum of all values from progenies of pure and aberrant *glomerata*	27, 28, 29 and 30	26	1	1	70,0 ± 37,2
		27	1 1 6 6 6 7 2 2	31	84,8 ± 6,7
		28	3 13 8 26 46 82 17 20 5 4 — 1	225	101,6 ± 2,5
		29	3 4 3 18 11 26 3 6 1 1 1 — 1	78	96,4 ± 4,2
		30	1 2 3 9 4 4 6 2 1	32	90,6 ± 6,6
		31	1 — — — 2 — 1	4	80,0 ± 18,6
		32	1 1	2	60,0 ± 26,3

The plants having exactly 28 chromosomes seem to be slightly more vigorous than the aberrants with lower and higher chromosome numbers, but the differences are not significant, the number of aberrants being too low.

A much greater material is represented by the progenies of aberrants with 27, 29 and 30 chromosomes. In these progenies the chromosome number is more variable and offers a better opportunity for studies of the relative vigour of plants with chromosome numbers from 26 to 32. In this case, too, plants with exactly 28 chromosomes represent the most vigorous category and the more the chromosome numbers depart from 28, on the plus as well as on the minus side, the lower are the average values (Table 8).

Since the progenies of pure and aberrant *glomerata* seem to be of the same type as regards the correlation between chromosome number and vigour, it may be correct to add all the values from the progenies of pure as well as aberrant *glomerata*. The result is given in the lower part of Table 8. As the chromosome numbers increase from 26 to 28 the average values also increase in the order 70,0—84,8—101,6. The 28-chromosome class represents the maximum, and in the higher classes (29—32) the average values again sink according to the series 96,4—90,6—80,0—60,0.

As the number of individuals, especially in the extreme classes, is rather limited, no differences are significant if two separate classes are compared. With a rather high degree of probability, however, the plants with 27 chromosomes are less vigorous than those with 28, the average values being $84{,}8 \pm 6{,}7$ and $101{,}6 \pm 2{,}5$. The D/m value in this case will be 2,33, the odds that this difference is significant being 51 to 1. However, if a series is formed of all aberrants with other chromosome numbers than 28, this series will have the average value $91{,}6 \pm 3{,}1$. The average value of the 28-chromosome class is $101{,}6 \pm 2{,}5$ and the difference between this and the former value $10{,}0 \pm 4{,}0$. Since the D/m value in this case will be 2,50 the odds that this difference is significant are 81 to 1. Hence it may be concluded that *in the material studied the plants with exactly 28 chromosomes are on the average more vigorous than plants with aberrant chromosome numbers.* This conclusion probably holds true of *Dactylis glomerata* in general, since the material studied is rather representative, consisting of eight progenies of pure *glomerata* and seven progenies of aberrants with 27, 29 and 30 chromosomes.

It was noted, however, that the effect of aberrant chromosome

numbers was different in different cases. In one of the progenies from 29-chromosome mother plants the individuals with 2n = 27 had a lower average vigour than the plants with 2n = 28, but the aberrants

TABLE 9. *Correlation between chromosome number and weight in the progeny of a pentasomic glomerata plant.*

Chromosome number	Weight: 0	100	200	300	400	500	600	700	800	900	1000	1100 gr.	n	M ± m
28	—	1	—	4	7	6	3	5	—	3	—	—	29	571 ± 36
29	1	1	3	10	6	3	2	2	—	—	1	—	29	436 ± 36
30	1	3	—	3	1	1	—	—	—	—	—	—	9	283 ± 65

with higher numbers (29 and 30) were equally or even more vigorous than the individuals with 28 chromosomes. The number of plants in this case, however, was too low to allow quite definite conclusions. In other progenies, on the other hand, the effect of aberrant chromosome

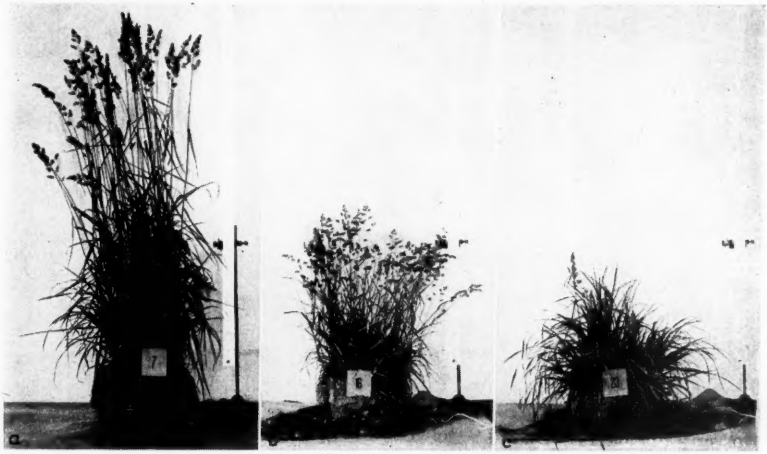


Fig. 69. Three trisomic individuals (2n = 27) with different vigour.

numbers was very marked. In the progeny 32—017 from a pentasomic mother plant (2n = 29) the average values (absolute weight) of the plants with 28, 29 and 30 chromosomes were 571 ± 36, 436 ± 36 and 283 ± 65 grammes respectively (Table 9). The difference between the

28 and 29 chromosome class gives the D/m value 2,65, the corresponding value for the difference between the 28 and 30 classes being 3,89. Also the plants with 29 and 30 chromosomes have probably a different average vigour, the D/m value in this case being 2,07. This progeny is the same as the one described in the preliminary report (MÜNTZING, 1933 a, Table 1).

The different effect of different chromosomes (homologous or non-homologous) is illustrated very well by the accompanying photos (Figs. 69—71). Fig. 69 represents three different *glomerata* individuals with $2n = 27$. The plant to the left (*a*) looks like an ordinary *D. glomerata* (cf. Fig. 67 *c*) but in the other plants (*b* and *c*) the lack of one chromosome is accompanied by a very marked reduction in vigour. Fig. 70 shows three different plants with $2n = 30$ from the

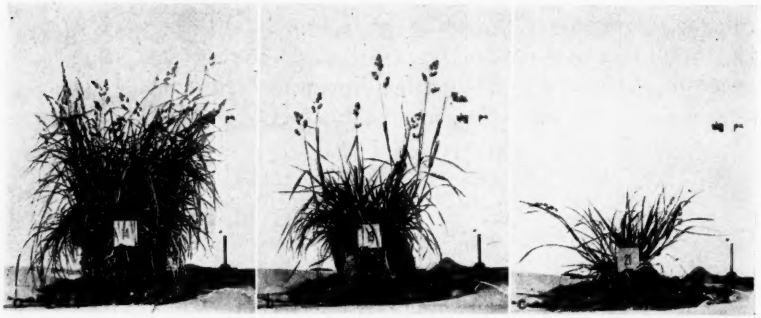


Fig. 70. Three plants with 30 chromosomes, differing in morphology and vigour.

progeny 32—017 (mentioned above). These plants, which may be directly compared with Figs. 67 and 69, are all less vigorous than normal *glomerata* but show different degrees of reduction. Fig. 71, finally, shows a plant with 29 chromosomes, which has almost normal vigour [1]. This plant is the mother of the progeny 32—017, to which all the plants in Figs. 69 and 70 belong (with the exception of 69 *b*).

Though there are average differences between the different chromosome classes, there is a still more striking variation in vigour within each class. This variation is probably due mainly to gene recombination, but in part it may be due to changes in the chromosomal constitution. In the 28 chromosome class not all the plants can be 4n individuals. Some of them may, for instance, have the constitution

[1] This photograph is reproduced on a larger scale than the other figures (compare the size of the measure).

4n — 1 + 1 and have arisen from the union of gametes with 13 and 15 chromosomes (involving non-disjunction in different quadrivalents). Judging from the meiotic observations most of the plants with 27 chromosomes are 4n — 1 plants, just as the 29-chromosome individuals generally have the formula 4n + 1. Of the plants with 30 chromosomes, those which have arisen in the progenies of 29-chromosome mother plants must as a rule correspond to the formula 4n + 2, but plants with 2n = 30, arisen directly from mother plants with 2n = 28,

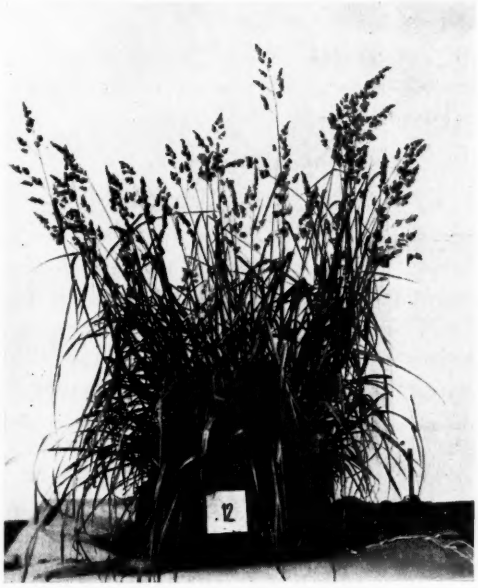


Fig. 71. A pentasomic plant (2n = 29).

are most likely 4n + 1 + 1 individuals. However, since the presence of an extra chromosome or the absence of one chromosome was not observed to influence the mode of association and disjunction in the other sets of four chromosomes, most of the plants with 28 chromosomes, arisen in the progeny of pentasomic and trisomic mother individuals, must be 4n plants, just as most of the 27- and 29-chromosome plants correspond to the formula 4n — 1 and 4n + 1. Thus, though there may occur some slight variation in the chromosomal constitution, most of the individuals in each chromosome class will correspond to the same formula. Hence the summation of all *glomerata-*

values available is justified, and the conclusions are based on comparable data.

Although there is a significant difference between the vigour of the plants with euploid and aneuploid chromosome numbers it should be emphasized, finally, that the differences found are rather slight in comparison with the violent effect of aneuploid chromosome numbers observed in the progenies of the cross triploid × *Aschersoniana*. The presence of one or a few extra chromosomes has a relatively slight effect, when the plants are in the 4n condition, and the average value is only lowered by 5,2 and 11,0 units respectively (Table 8). The absence of one or two chromosomes, however, causes a more severe disturbance, the depression of the average value in this case being 16,8 and 31,6 units respectively.

5. TRIPLOID × GLOMERATA.

The correlation between chromosome number and relative vigour in the progenies obtained from the cross triploid × *glomerata* is represented in Table 10. As in this case the chromosome variation is very great, occasional slight errors in the determination of the chromosome numbers will have very little effect on the degree of correlation. Therefore, when making up the table, the »total» values were used (accurate counts as well as ± values). Unfortunately, the number of individuals in most classes is rather low, but some definite conclusions may nevertheless be drawn. Considering first the upper part of Table 10 (the lower chromosome numbers), *there is a rather clear maximum at the number 28*. As the chromosome numbers increase from 25 to 28, the average values increase from 73,8 to 129,0. The difference between the average values of the 28- and 27-class is $25{,}6 \pm 9{,}2$ ($D/m = 2{,}78$), between the 28- and 26-class $54{,}6 \pm 10{,}2$ ($D/m = 5{,}35$) and between the 28- and the 25-class, finally, $55{,}2 \pm 13{,}4$ ($D/m = 4{,}12$). All these differences may thus be considered to be significant. The lowest chromosome numbers in the table, 24 and 22, (which approach the triploid number 21) seem to represent another increase in vigour, but the differences between these average values (80,0 and 90,0) on one hand and the average values of the 25- and 26-class on the other (73,8 and 74,4) are not significant.

As regards the lower part of the table, representing the chromosome numbers 29—41, the number of individuals in each class is too low to admit any sure conclusions being drawn. However, if the variants with chromosome numbers ranging from 28 to 36 are com-

TABLE 10. *Triploid × glomerata. Correlation between chromosome number and relative vigour.*

Chromosome number	Dead	Relative vigour: 0	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	340	n	M ± m
22						1	1	1												3	90,0 ± 26,4
24				1	—	—	—	—	1											2	80,0 ± 32,4
25	1			2	5	4	1	2	1	1										16	73,8 ± 11,4
26	2	2		7	8	8	6	1	5	1	2									40	74,4 ± 7,2
27		1		7	4	6	11	9	13	5	6	—	—	1						63	103,4 ± 5,8
28				1	2	5	4	4	10	6	5	1	1	1	—	—	—	—	1	41	129,0 ± 7,1
29				2	—	—	2	6	1											11	93,6 ± 13,8
30							1	1	—	—	1									3	123,4 ± 26,4
31							2	—	1											3	103,4 ± 26,4
32						1	—	—	1											2	100,0 ± 32,4
33		1		2	1	3	1	—	—	—	—	1								9	67,8 ± 15,2
34							1	2	2											5	114,0 ± 20,4
35	1	1		1	—	1	1	1	2	2	1									10	104,0 ± 14,5
36					1	—	—	1	1	1										4	110,0 ± 22,8
37				1																1	30,0 ± 45,8
38	1																			—	—
40	3																			—	—
41									1											1	130,0 ± 45,8

bined into three classes instead of nine the following values will be obtained. The series formed by the variants with 28, 29 and 30 chromosomes will have the average value $121{,}6 \pm 6{,}2$, the corresponding value of the next class (variants with 31, 32 and 33 chromosomes) will be $80{,}0 \pm 12{,}2$ and of the third class (34, 35 and 36), finally, $107{,}8 \pm 10{,}5$. The difference between the first and second of these classes is $41{,}6 \pm \pm 13{,}6$ ($D/m = 2{,}97$) and is consequently significant. The difference between the second and third class is not significant ($D/m = 1{,}72$). These data, however, suffice to show that also above 28 there is a

Fig. 72. From left to right: Greenhouse plants with 14 (*D. Aschersoniana*), 28 (*D. glomerata*) and 35 chromosomes. The last-mentioned plant obtained from the back cross spont. triploid × *glomerata*.

decrease in vigour. It is also probable that there is a second maximum corresponding to the pentaploid chromosome number 35.

Some of the ± pentaploid plants are very vigorous and clearly more robust than the mother clone of the commercial variety Skandia II, used for comparison. This is evident from Fig. 72, which shows greenhouse plants of *D. Aschersoniana* (*a*), *glomerata*, Skandia II (*b*) and a pentaploid derivative (*c*). The average value of the pentaploids, however, is lower than that of the 28-chromosome class. Some pentaploids were quite weak, causing a marked decrease in the average value, and on the other hand some 28-chromosome plants were remarkably vigorous. It seems probable, however, that there is a greater chance to obtain plants with maximal vigour among the pentaploids than

among the segregates with 28 chromosomes. — The single exceptional plant with 56 chromosomes (cf. above p. 144) was very weak and died in spite of all efforts to keep it alive. This plant seems to have exceeded the optimal chromosome number.

On the whole there is an excellent correspondance between the average values of the present material and those obtained from progenies of pure and aberrant *glomerata* (Table 8). In both cases the 28-class represents a maximum, and in both cases the chromosome numbers below 28 are correlated with a marked decrease in vigour. In both cases a decrease in the average weight is also characteristic of the classes just above 28 (29—32), but this decrease is less pronounced than in the classes below 28. This similarity in behaviour indicates a similarity or homology of the chromosome constitution in the different classes.

Such a homology is indeed to be expected from the meiotic behaviour of the triploid hybrids. As reported above in detail (p. 136) there are seven sets of three homologous chromosomes in the *glomerata* × *Aschersoniana* hybrids, which may be indicated by $a_1a_2a_3$, $b_1b_2b_3$ . . . $g_1g_2g_3$. At meiosis the chromosomes in each set are present either as a trivalent or as a bivalent + a univalent. Consequently all gametes will receive either one or two representatives of each set. In *Dactylis glomerata* there must be seven sets of four homologous chromosomes ($a_1a_2a_3a_4$, $b_1b_2b_3b_4$ . . . $g_1g_2g_3g_4$) and thus almost all of the gametes will contain two representatives of each set. If a plant with 28 chromosomes is obtained from the cross triploid × *glomerata*, this will in most cases be a 4n plant with four homologous chromosomes of each kind, since gametes with 14 chromosomes, produced by the triploid, will contain two chromosomes of each kind, just as the male *glomerata* gametes. For the same reasons a plant with 27 chromosomes will correspond to the formula 4n — 1, one of the seven sets in the 13-chromosome female gamete being represented by only one chromosome, the other sets by two. With regard to the plants with higher numbers than 28 those with 29 chromosomes may be assumed to have arisen through the functioning of male gametes with 15 chromosomes. It was shown above that such gametes really do function (p. 135). On this assumption the plants with 29 chromosomes, obtained in the progeny of triploids, will have the same constitution (4n + 1) as those occurring in *D. glomerata*. The plants with 30—34 chromosomes, obtained in the back cross, have been assumed to be the result of a union between reduced *glomerata* gametes and ovules which are almost un-

reduced, but in which one or more chromosomes have been eliminated. It is more likely that the eliminated chromosomes will belong to different sets rather than to the same set, and therefore the formula of plants with, e. g. 30, 31 and 32 chromosomes will be $4n + 1 + 1$, $4n + 1 + 1 + 1$ and $5n - 1 - 1 - 1$ rather than $4n + 2$, $4n + 3$ and $5 - 3$. Most of the *glomerata* aberrants with $2n = 30$ (at least those which are daughters of pentasomic mother plants) are probably $4n + 2$ individuals. However, the average decrease in vigour seems to be about the same, whether the extra chromosomes belong to the same or to different sets.

6. PROGENIES FROM PENTAPLOIDS.

As will be further discussed below the progenies obtained after self-fertilisation of pentaploids are, on the average, very poor but also very variable. This is indeed to be expected, since most of the plants in these progenies have aneuploid chromosome numbers, ranging from 29 to 41. As regards the correlation between chromosome number and vigour the data obtained are given in Table 11. Since the number of individuals in each class is rather low the differences between the average values of the separate classes are not significant. However, as there seemed to be a minimum between 28 and 35, the values were added to give the triple classes represented in the lower part of Table 11. Considering first the variants with 28—36 chromosomes, the first big class ($28 + 29 + 30$) has the average value $109{,}4 \pm 19{,}5$, the corresponding values of the second and third class being $74{,}3 \pm 9{,}8$ and $105{,}6 \pm 9{,}6$. Thus the plants with the intermediate chromosome numbers 31—33 seem to be less vigorous than those with 28—30 or 34—36 chromosomes. The difference $105{,}6 - 74{,}3$ is the only possibly significant one, however, the D/m value in this case being 2,28. If the two outer classes (28—30 and 34—36) are added the average value of this series will be $106{,}4 \pm 8{,}6$. The difference between this value and that of the intermediate class (31—33) will be $32{,}1 \pm 13{,}0$ and D/m 2,47. The odds that this difference is significant are 75 to 1. Thus, it is highly probable that the daughter plants of pentaploids with the intermediate chromosome numbers 31—33 are less vigorous than plants with euploid or approximately euploid numbers.

Meiosis in the pentaploids was rather irregular (p. 147), but since multivalents were rather frequent most of the gametes will receive either two or three representatives of the seven chromosome sets. Though many of the daughter plants with 35 or 28 chromosomes may

TABLE 11. *Progenies of pentaploids. Correlation between chromosome number and relative vigour.*

Chromosome number	Dead	Percentage of dead plants	Relative vigour: 0	40	80	120	160	200	240	280	320	360	400	440	480	n	M ± m
28	0	0						1	—	1						2	220,0 ± 56,8
29	0	0	1	1	—	1										3	73,2 ± 46,4
30	0	0	1	2	6	2	1									12	190,0 ± 23,2
31	0	0	4	4	2	3	2									15	86,8 ± 20,8
32	5	16	13	9	—	4	1									27	57,2 ± 15,5
33	4	14	12	4	2	2	2	1	—	2						25	85,6 ± 16,1
34	8	24	6	4	6	2	1	2	1	1	1	—	—	1		25	128,8 ± 16,1
35	11	27	11	5	3	3	6	—	1	—	1					30	97,2 ± 14,7
36	2	11	5	4	2	2	3									16	85,0 ± 20,1
37	0	0	2	1	2	—	1									6	80,0 ± 32,8
38	0	0	3	2	1	—	—	—	1	—	1					8	110,0 ± 28,4
39	0	0	1	—	1											2	60,0 ± 56,8
41	0	0									1					1	300,0 ± 80,4
28 + 29 + 30	0	0,0 ± 9,3	2	3	6	3	2	—	1							17	109,4 ± 19,5
31 + 32 + 33	9	11,8 ± 4,4	29	17	4	9	5	1	—	2						67	74,3 ± 9,8
34 + 35 + 36	21	22,8 ± 4,0	22	13	11	7	10	2	2	1	2	—	—	1		71	105,6 ± 9,6
37 + 38 + 39	0	0,0 ± 9,6	6	3	4	—	1	—	1	—	1					16	92,4 ± 20,1

be unbalanced, some of them may have the formula 5n or 4n exactly or approximately. *All* plants with intermediate chromosome numbers, however, must be more or less unbalanced, and hence it is comprehensible that such numbers are correlated with a decrease in vigour.

The mortality in the present material was on the average very great (Table 11), but the percentage of dead plants seems to be highest in the 34—36 class. A calculation of the standard errors on the basis of the average percentage of dead plants (18) shows, however, that the differences are not significant, though it is rather probable that the mortality is lower in the 28—30 class than in the 34—36 class ($D/m = 2{,}28$).

7. COMBINATION OF ALL DATA CONCERNING CHROMOSOME NUMBER AND RELATIVE VIGOUR.

An effort has been made to combine all the data available into a single series. The result of this effort is represented in Diagram 1, showing the correlation between chromosome number and relative vigour with regard to the entire interval 14—37.

Though some higher chromosome numbers were obtained, they are too few and their vigour values too unreliable to be included in the diagram. The other values were obtained in the following way: 1) The series given in Table 8 (*D. glomerata*) and Table 10 (triploid × *glomerata*) were added, and the new average values of the classes involved (26—32) were calculated. 2) The values in Table 11 (progenies from pentaploids) were combined with the preceding average values. This was done by multiplying the two average values of each chromosome class with the corresponding number of variates by adding the two products and by dividing the sum by the total number of variates. For instance, in the 36-chromosome class in Table 10 there are four variates with the average value 110,0, in the same class in Table 11 there are 16 variates with the average value 85,0. Four times $110{,}0 + 16 \times 85{,}0$ is equal to 1800 and this value divided by 20 is 90,0, which represents the final value of the 36 class. In this way the values for the interval 22 to 37 were obtained. The values of the 21- and 14-classes were obtained by a consideration of Table 7 (plant weight of *Aschersoniana*, triploid hybrids and *glomerata*). If the average values of the 28, 21 and 14 classes are assumed to correspond to the ratio 572 : 467 : 175, the final values of the 21- and 14-classes will be 87,1 and 32,6, since the final value of the 28-class had previously been found to be 106,7.

Finally, the relative values of the classes 15 to 20 could be obtained by making the rather plausible assumption that the vigour of the segregation product with 14 chromosomes, obtained from the cross triploid × *Aschersoniana*, is approximately the same as the average value of *Aschersoniana*. The weight relations between the 14 chromosome plant and the aneuploid classes (15 to 20) have been given above (p. 162). As the final value of *Aschersoniana* (class 14) was found to be 32,6, the corresponding value of the 15 chromosome class will be $(100 \cdot 32{,}6) : 475 = 6{,}9$, and

of the 16 chromosome class $(25 . 32{,}6) : 475 = 1{,}7$. The remaining values were calculated in the same way.

The curve (Diagram 1) represents the following values, obtained in the manner described above (the chromosome classes in brackets): 32,6 (14); 6,9 (15); 1,7 (16); 2,9 (17); 1,7 (18); 5,1 (19); 8,6 (20); 87,1 (21); 90,0 (22); 80,0 (24); 73,8 (25);

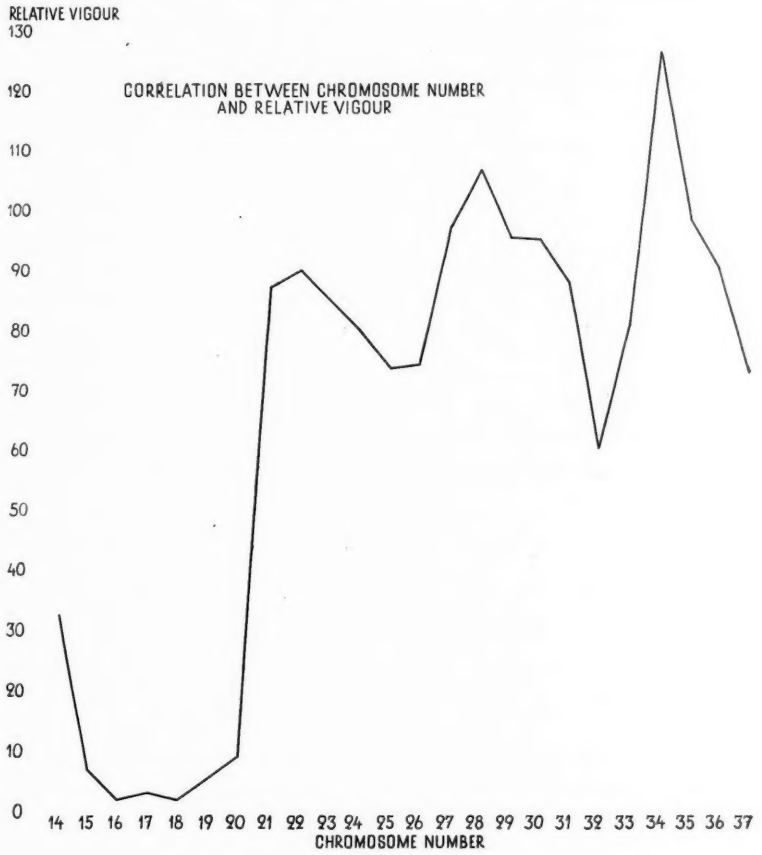


Diagram 1.

74,4 (26); 97,2 (27); 106,7 (28); 95,3 (29); 95,1 (30); 87,8 (31); 60,1 (32); 80,9 (33); 126,3 (34); 98,9 (35); 90,0 (36); 72,9 (37).

Among the weak points in this diagram the low number of individuals in some classes should be pointed out first, the values being based on less than ten variants in the following classes: 15—24 and 37. Sec-

ondly, the combination of the data from the progenies of pentaploids with the rest of the material causes the values above 28 to be too high in relation to the lower numbers, since the average weight of the pentaploid progenies was rather low. Thirdly, the values of the 21- and 14-classes are probably too low in comparison with the 28-class (cf. above p. 160) and this in its turn makes the 15—20 values somewhat more depressed than they probably should be.

In spite of these and other inaccuracies the curve expresses the following facts:

1) *The euploid numbers 14, 21 and 28 represent maxima of increasing size, the plants with 28 chromosomes being at least twice as vigorous as the plants with 14 chromosomes and the triploids with 21 chromosomes being intermediate. With a high degree of probability there is a fourth maximum at 35 (or approximately that number) but the average vigour of these plants is not significantly greater than that of the plants with 28 chromosomes.*

2) *The aneuploid numbers 15 to 20 represent a very deep minimum, the plants with 16—18 chromosomes scarcely being viable. A second minimum, situated between 21 and 28, is rather marked but less extreme than the first one. A third, less clear minimum is situated between 28 and 35, and above the pentaploid number the curve probably declines once more.*

8. SOME ABSOLUTE VALUES.

In order to elucidate some points, which have not been considered above, the absolute values of some different types of progenies may be given (Table 12). On account of the limited space available it has not been possible to publish the distributions but only the average values. Table 12 contains three sets of data, indicated by the field numbers 1935—1 to 1935—21, 35—02 to 35—07 and 36—1 to 36—13 respectively. Within each of these groups the average values are comparable, being based on the sum of 3, 2 and 1 weighings respectively. Starting with the first group, a comparison between the average values of the progenies 1935—1 to 1935—21 reveals the following facts.

The first five numbers (1935—1 to 1935—5) represent *progenies of pentaploids* and *are characterized by a very poor average vigour and a very high percentage of dead plants.* (Most of these plants died in the field during the winter 1935—1936). This is especially evident from a comparison with 1935—21, a progeny of ordinary *D. glomerata*, obtained by self-fertilisation of a Skandia II plant (from the mother clone). The average value of this progeny is 686 ± 36 gr., the average of the best pentaploid progeny being only 270 gr. The percentage of dead plants was only 2 in the *glomerata* progeny but varied between 23 and 44 in the pentaploid families.

Other progenies from pentaploids have been raised, however, which seem to be more successful. Among the pentaploid families in the second group in Table 12

TABLE 12. *Average plant weight in different Dactylis progenies.*

Field number	Kind of progeny	Number of weighings	Chromosome number of the mother plant	n	M ± m (gr.)	σ	v	Number of dead plants	Percentage of dead plants
1935 — 1	pentaploid, selfed	3	± 34	20	270	—	—	6	23
— 2	» »	»	36	17	158	—	—	10	37
— 3	2 pentaploids, crossed	»	36 × 36	11	154	—	—	5	31
— 4	pentaploid, selfed	»	36	17	170	—	—	13	43
— 5	» »	»	± 35	20	180	—	—	16	44
1935 — 6 + 7	triploid × *glomerata*	»	21	7	614	—	—	0	0
— 8 + 9	» » »	»	»	74	430 ± 26	222	52	4	5
— 10 + 11	» » »	»	»	57	602 ± 50	378	63	1	2
— 12	» » »	»	»	5	660	—	—	0	0
1935 — 13	*glomerata*-aberrant, selfed	»	27	5	260	—	—	1	17
— 14	»	»	»	41	330 ± 27	171	52	0	0
— 15	»	»	»	7	414	—	—	0	0
— 16	»	»	29	49	398 ± 25	174	44	0	0
— 17	»	»	»	48	842 ± 62	429	51	1	2
— 18	»	»	30	40	710 ± 51	320	45	0	0
— 20	»	»	»	5	180	—	—	1	17
1935 — 21	Skandia II, selfed	»	28	45	686 ± 36	243	35	1	2
35 — 02	pentaploid, selfed;	2	36	25	99,0 ± 15,4	77	78	4	14
— 03	the same pentaploid, crossed	»	»	44	165,0 ± 15,7	104	63	4	8
— 07 a	Skandia II, commercial variety	»	28	30	190,0 ± 18,9	104	55	0	0
— 04	pentaploid, selfed;	»	35	63	80,5 ± 8,3	66	82	5	7
— 05	the same pentaploid, crossed	»	»	146	139,5 ± 7,2	87	62	4	3
— 06	pentaploid, selfed	»	34	11	61,5	—	—	4	27
— 07 b	Skandia II, commercial variety	»	28	53	156,0 ± 9,0	67	43	0	0
36 — 1	triploid × *glomerata*	1	21	28	148,0 ± 13,5	71	48	0	0
— 2	» » »	»	»	23	164,0 ± 13,7	66	40	0	0
— 3	» » »	»	»	9	253,0	—	—	1	10
— 13 a	Skandia II, comm. var.	»	28	20	190,0 ± 16,3	73	38	0	0
— 4	pentaploid, selfed;	»	35	78	144,0 ± 7,7	68	47	2	3
— 5	the same pentaploid crossed	»	»	74	167,5 ± 9,8	84	50	1	1
— 13 b	Skandia II, comm. var.	»	28	20	167,5 ± 12,2	55	33	0	0

Field number	Kind of progeny	Number of weighings	Chromosome number of the mother plant	n	M ± m (gr.)	σ	v	Number of dead plants	Percentage of dead plants
36 — 6	pentaploid, crossed		36	75	85,5 ± 5,5	48	56	0	0
— 7	» »		»	68	48,5 ± 4,2	35	72	7	9
— 13 c	Skandia II, comm. var.		28	20	137,5 ± 15,4	69	50	0	0
— 8	pentaploid, crossed		± 34	29	83,5 ± 7,2	39	47	1	3
— 9	» »		± 35	29	80,0 ± 8,0	43	54	1	3
— 10	» »		35	30	118,5 ± 13,1	72	61	0	0
— 11	» »		± 35	29	125,0 ± 13,4	72	58	1	3
— 12	» »		± 34	30	113,5 ± 10,9	60	53	0	0
— 13 d	Skandia II, comm. var.		28	28	152,0 ± 11,4	60	39	0	0

the best average values are represented by the families 03 with 165,0 ± 15,7 gr. and 05 with 139,5 ± 7,2 gr. In this group plants from commercial seed of Skandia II were used as a standard, the average values of two such families (07 and 07 b) being 190,0 ± 18,9 and 156,0 ± 9,0 respectively. These values are only slightly better than those of the best pentaploid families. However, the other pentaploid families in the same group (02, 04 and 06) were quite inferior in vigour.

The same situation is again met with in the third group of families (36—1 to 36—13). Some of the progenies of pentaploids are rather good, e. g. 36—4, —5 and —11 (the average value of 36—5 being exactly the same as that of the adjacent standard family). In this group of families, too, most of the pentaploid progenies were more or less poor (e. g. 36—6 and —7).

When comparing the average values it is quite necessary to note whether the mother plants have been selfed or crossed. In three cases, in which the same mother individual (different clone plants) had been used for selfing and for crossing, *the average value of the crossed family was higher than that of the selfed.* The following average values were obtained: 1) 35—02 and 03, 99,0 ± 15,4 (selfed) and 165,0 ± 15,7 (crossed); 2) 35—04 and 05, 80,5 ± 8,3 (selfed) and 139,5 ± 7,2 (crossed); 3) 36—4 and —5, 144,0 ± 7,7 (selfed) and 167,5 ± 9,8 (crossed). Especially in the first two cases the differences are very great, indicating a rather marked inbreeding degeneration. It should be mentioned that the crosses were simply performed by placing two or more plants together in an isolation cage or a special closed part of the greenhouse. Some plants considered to be cross-fertilized may in reality be partly or totally self-fertilized, since there are many different degrees of self-fertility in *Dactylis* (SYLVÉN, 1929). This may account for the relatively slight differences between the families 36—4 and —5 and for the poor vigour of some families derived from mother plants presumably cross-fertilized.

The second category of progenies represented in Table 12 are those obtained from the crosses triploid × *glomerata*. The average values of these families (1935 —1 to 1935—12 and 36—1 to 36—3) are evidently rather good, much better than those of the pentaploid families. This is certainly due to the fact that, approximately or exactly, they have the euploid chromosome numbers 28 and 35.

Some of the 1935 families, finally, are progenies from *glomerata*-aberrants with 27, 29 and 30 chromosomes. The average values may be very different, even if the chromosome number of the mother plant is the same. This shows that *parallel with the influences of different chromosome numbers the genetical constitution of the mother plant is of prime importance for the vigour of the progeny.* The family 1935—18 is remarkable in having a rather high average value (710 ± 51), though most of the plants have chromosome numbers ranging from 29 to 32. The chromosome variation of this progeny is given in Table 3 under the field number D. 9 [1].

The percentage of plants which died after transplantation to the field has been calculated for all the progenies in Table 12. As already pointed out this percentage was very high in the progenies of pentaploids, especially in the families 1935—1 to —5, which were germinated in the spring of 1935 and transplanted to the field in the beginning of the summer. Some of these plants must have died during dry periods in the summer, others in the winter 1935—1936. The average percentage of dead plants is lower in the families 35—02 to —06, which are germinated in August 1935 and transplanted to the field in the autumn. The losses in this case are mainly due to bad wintering. The 1936 families have not yet wintered and the percentage of dead plants is therefore still lower than in the other two categories. — It is striking that the progenies of pure *glomerata,* used as standards are almost free from dead plants. The triploid $\times$ *glomerata* families and the *glomerata* aberrants have also a low percentage of losses.

Finally, it should be mentioned that the coefficient of variation (v) has been calculated for all the progenies. Though the significance of this value is doubtful, it is striking that almost without exception the lowest values of this coefficient were found among the progenies of pure *glomerata,* in which chromosome variation is at a minimum. All the other progenies, which are known to have a considerable chromosomal variation, are also characterized in having high coefficients of variation. This is in accordance with the observed and very conspicuous morphological variation in these progenies.

IV. CHROMOSOME NUMBER AND FERTILITY.

1. METHODS.

Fertility has been studied chiefly by determinations of the percentage of apparently good pollen. The pollen samples were mounted in a mixture of glycerin and aceto-carmine, and in the majority of the cases the percentage of good pollen was determined by counts of at least 200 grains. In several cases more than one sample was taken from the same plant, either in the same year or in different years. In such cases the average value of all determinations is used in the tables. In 1936 the percentage values (class width = 10 per cent) were determined by mere gradation, but special control series showed that this was quite a reliable method. For this work I am much indebted to Mr. B. ROSENBERG and Mr. H. N. JOHNSSON.

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[1] In order to facilitate further comparison between the absolute values in Table 11 and the chromosome numbers in Table 3 it should be mentioned that 1935—13 corresponds to D. 2, 1935—14 to D. 4, 1935—15 to D. 6, 1935—16 to D. 7, 1935—17 to D. 8 and 1935—20 to 017—22 a.

To some extent the female fertility (after open pollination) was determined by counting the number of seeds per a certain weight of panicle substance. In most cases 500 mg. were examined. Since this weight also, and sometimes chiefly, represents the weight of the seeds, a correction was made afterwards in order to get comparable data. From the reports of the works of the Swedish State Seed Testing Station (WITTE, 1927—1936) it was seen that the average weight of one seed of *Dactylis glomerata* is almost exactly one milligram. The same value was obtained by a calculation of the average weight of 20 samples, taken at random from my own material. Thus, all values were corrected on the basis of this seed weight, and after this correlation the data obtained should be quite comparable. The values given in Tables 14 and 16 represent the number of seeds per 1 gr. of flower substance.

2. D. ASCHERSONIANA.

A report of the pollen fertility in *D. Aschersoniana* has already been given above (pp. 118—119) in connection with a survey of the meiotic conditions. In this connection it need only be repeated that most biotypes of the species, which have regular meiosis, are evidently quite pollen fertile, the percentage of good pollen generally being higher than 90. Since a few *Aschersoniana* plants were partially sterile, the average value of all counts in this species was found to be somewhat lower, the value in question being 84,0.

3. SPONTANEOUS TRIPLOID HYBRIDS.

As already mentioned (p. 136), all triploid *glomerata* × *Aschersoniana* hybrids observed are completely male sterile with non-dehiscing anthers. The pollen quality in these closed anthers was quite bad. By crushing the anthers it was possible to determine the percentage of good pollen, which was found to vary between 4 and 10. In seven preparations from four different hybrids the following values were obtained: 10, 10, 8, 8, 4, 6 and 7. In this case as in many other similar cases, there is evidently a positive correlation between the percentage of good pollen and the ability to dehisce.

4. TRIPLOID × ASCHERSONIANA.

Of the plants obtained from the back cross triploid × *Aschersoniana* only the solitary plant with 14 chromosomes had good fertility. All the other aneuploid plants were either completely male sterile with non-dehiscing anthers or did not even produce panicles With regard to these characters, and as the plants were observed in two summers, four different categories could be distinguished. The data available may be arranged in the following way:

Category number:	1	2	3	4
Degree of sterility:	Only vegetative development, no panicles either in 1935 or 1936	Panicles only in one of the years, then completely male sterile	Panicles in both years, completely male sterile	Panicles in both years, the anthers open but pollen partially sterile
Chromosome numbers:	17 (dead in 1936)	17 (no anthers)	15	14
	18	17	15	
	18	17	16	
		20	19	
			20	
			20	

The four categories described above may be considered to represent different degrees of sterility, though the viability of the plants is also taken into consideration. Since *all* the plants with 17 or 18 chromosomes belong to categories 1 or 2, it is evident that *the most intermediate chromosome numbers not only represent the poorest viability but also the poorest fertility* (if the latter conception is taken in a wide sense). On the other hand four of the five plants, having either 15 $(2n + 1)$ or 20 $(3n - 1)$ chromosomes belong to the relatively good category 3, and the only pollen producing plant (category 4) was euploid, having $2n = 14$ like *D. Aschersoniana.* Not even this plant, however, had quite good pollen fertility but was found to be about semi-sterile. In addition to this partial pollen sterility the panicles of this plant had a marked tendency (especially under greenhouse conditions) to become white and die shortly after the flowering.

5. D. GLOMERATA.

A. POLLEN FERTILITY.

In spite of the presence of a high number of quadrivalents at meiosis pollen fertility in *D. glomerata* is generally perfectly good. E. g. in »Skandia II», the type chiefly used for the meiotic observations, the following percentages of good pollen were found in the years 1934—1936: 88, 95, 95, 94, 94, 96 (1934); 98 (1935) and 90, 90 (1936). The same good fertility is generally characteristic of *glomerata* types with 28 chromosomes, though in exceptional cases the percentage of good pollen may be lower.

The values obtained from progenies of pure *glomerata* are summarized in the upper part of Table 13. The mean value of the 28-chromosome class is 89,2. The aberrants with 27, 29 and 30 chromo-

TABLE 13. *Dactylis glomerata. Correlation between chromosome number and pollen fertility.*

Kind of progeny	Chromosome number of the mother plants	Chromosome numbers in the progeny	Male sterile plants		Per cent apparently good pollen:								n	M ± m
			number	per cent	30	40	50	60	70	80	90	100		
Progenies of pure *glomerata*	28	27							2	—	1	5	8	86,3
		28	1	(1)			1	2	2	5	16	52	78	89,2
		29								2	2	3	7	86,4
		30										1	1	(95,0)
Progenies of *glomerata* aberrants	27, 29 and 30	26						1	—	—	—	1	2	75,0
		27	4	15				4	3	9	3	3	22	74,1
		28	4	6	1	—		1	9	10	17	23	61	82,9
		29	8	17			1	3	6	16	7	6	39	76,0
		30	2	7			1	—	7	4	6	9	27	80,2
		31	1	(20)					1	1	2		4	77,5
		32							1				1	(65,0)
Sum of all values from progenies of pure and aberrant *glomerata*	27, 28, 29 and 30	26						1	—	—	—	1	2	75,0 ± 8,9
		27	4	12				4	5	9	4	8	30	77,3 ± 2,3
		28	5	3	1	1	1	3	11	15	33	75	139	86,4 ± 1,1
		29	8	15			1	3	6	18	9	9	46	77,6 ± 1,9
		30	2	7			1	—	7	4	6	10	28	80,7 ± 2,4
		31	1	(20)					1	1	2		4	77,5 ± 6,3
		32							1				1	65,0 ± 12,6

somes have also rather good pollen, but on the average their pollen fertility seems to be somewhat lower than that of the 28-chromosome class. However, the differences in this case are not significant. More extensive data were obtained from the progenies of *glomerata* aberrants, and significant differences were obtained from the sum of all values from the progenies of pure and aberrant *glomerata* (Table 13). This material is the same as that employed for the study of the correlation between chromosome number and vigour. The average pollen fertility of all 28-chromosome *glomerata* plants studied is 86,4 ± 1,1 per cent, and this value is higher than all the means of the aneuploid classes. If all the aneuploid classes are added to one series the average value of this series will be 74,5 ± 1,2. The difference, 86,4 — 74,5 is 11,9 ± 1,6 and D/m = 7,44. Thus, *in Dactylis glomerata plants with the typical chromosome number 28 have on the average better pollen fertility than aberrants with aneuploid numbers.*

Significant differences are also obtained if the means of the different chromosome classes are compared. The difference between the 28- and 27-classes is $9{,}1 \pm 2{,}5$ ($D/m = 3{,}64$), between the 28- and 29-classes $8{,}8 \pm 2{,}2$ ($D/m = 4{,}00$) and between the 28- and 30-classes $5{,}7 \pm 2{,}6$ ($D/m = 2{,}19$).

In addition to the plants with normal pollen production there was also a small proportion of male sterile plants with non-dehiscing anthers. Such plants were found to be more numerous among the aneuploid plants than in the 28-chromosome class. In the entire material studied there were 20 male sterile individuals (Table 13) among a total of 270 plants, which corresponds to 7,41 per cent. The percentage of male sterile plants was found to be $3{,}47 \pm 2{,}18$ in the 28-chromosome class, and, on an average, $11{,}90 \pm 2{,}33$ in the aneuploid classes. The difference between these values is $8{,}43 \pm 3{,}19$ and $D/m = 2{,}64$. Thus with a high degree of probability there is a positive correlation between aneuploidy and the occurrence of plants with non-dehiscing anthers.

B. SEED SETTING.

The degree of seed-setting after open pollination may be considered to represent a rough measure of the female fertility. In progenies of

TABLE 14. *Seed setting in progenies of pure and aberrant Dactylis glomerata.*

Chromosome number	Number of seeds per 1 gr. of panicle substance													n	M ± m
	0	500	1000	1500	2000	2500	3000	3500	4000	4500	5000	5500	6000	6500	
27	11	6	1	2	—	1									21	703 ± 215
28	14	19	9	10	5	4	2	1	—	1					65	1289 ± 122
29	9	6	3	—	—	—	—	1	—	—	—	—	1		20	1025 ± 220
30	5	8	1	2											16	750 ± 246
31	1	1	—	1											3	915 ± 570
32	1														1	250 ± 985

pure and aberrant *glomerata* the above listed values were obtained (Table 14). As is evident from the table the variation is very great and the differences between the average values of the different classes are not significant. However, the plants with 28 chromosomes have the highest average value, 1289 ± 122 seeds per gramme of panicle substance, and the total average value of all the aneuploid classes was

found to be 825 ± 126. The difference between these values is 464 ± 175 and $D/m = 2{,}65$. Thus, *also with regard to the female fertility the plants with the typical chromosome number of glomerata are superior to the aneuploid aberrants.*

6. TRIPLOID × GLOMERATA.

A. POLLEN FERTILITY.

In the material obtained from the cross triploid hybrids × *glomerata* a study of the correlation between pollen fertility and chromosome number gave the following data, summarized in Table 15. As

TABLE 15. *Triploid × glomerata. Correlation between chromosome number and pollen fertility.*

Chromosome number	Male sterile	Percentage of good pollen: 20	30	40	50	60	70	80	90	100	n	M ± m
22	1		1	1							2	$30{,}0 \pm 11{,}7$
24	1										—	—
25	4			1	—	—	5	4	—	1	11	$68{,}6 \pm 5{,}0$
26	6		1	3	1	6	4	6	8	5	34	$69{,}7 \pm 2{,}8$
27	4			1	—	12	10	9	11	18	61	$76{,}5 \pm 2{,}1$
28	3			1	—	2	1	6	4	24	38	$86{,}3 \pm 2{,}7$
29	0					3	1	2	—	5	11	$77{,}7 \pm 5{,}0$
30	0					1	—	—	—	3	4	85,0
31	0								1	1	2	90,0
32	0									2	2	95,0
33	4						1	—	1	3	5	87,0
34	0							1	—	6	7	92,1
35	0								2	9	11	93,2
36	1							1	1	7	9	91,7
37	0								1		1	(85,0)
41	0								1		1	(85,0)

is evident from these values *the low chromosome numbers are characterized by partial pollen sterility, the high numbers by almost complete fertility.* In the interval 22 to 28 there is a very marked positive correlation between chromosome number and pollen fertility, the average values increasing from $30{,}0 \pm 11{,}7$ to $86{,}3 \pm 2{,}7$ and the differences between several of the individual classes being significant. Thus, the difference between the 28- and 27-classes is $9{,}8 \pm 3{,}4$ ($D/m = 2{,}88$), between 28 and 26 $16{,}6 \pm 3{,}9$ ($D/m = 4{,}26$), between 28

and 25 $17{,}7 \pm 5{,}7$ ($D/m = 3{,}11$) and between 28 and 22, finally, $56{,}3 \pm 12{,}0$ ($D/m = 4{,}69$).

Above 28 there is no significant decrease in fertility, though the 29- and 30-classes seem to have somewhat lower values. Above 30 the maximum fertility seems to be attained, most of the material having 90—100 per cent good pollen. — A low frequency of completely male sterile plants was also met with in the present material, the percentage of such plants being especially high in the classes with the lowest chromosome numbers (Table 15).

A comparison between Tables 15 and 13 shows a close correspondance between the average values of the chromosome classes which these two groups of material have in common. The 28-chromosome class of the *glomerata* plants had the value $86{,}4 \pm 1{,}1$, the corresponding value of the triploid × *glomerata* plants being $86{,}3 \pm 2{,}7$. In the 27-classes the two mean values obtained were $77{,}3 \pm 2{,}3$ and $76{,}5 \pm 2{,}1$ respectively, and among the plants with 29 chromosomes those of *glomerata* had the average value $77{,}6 \pm 1{,}9$, those of triploid × *glomerata* $77{,}7 \pm 5{,}0$. This very close correspondance strongly indicates a constitutional similarity. The same conclusion was drawn from the study of relative vigour (cf. above p. 171).

TABLE 16. *Seed setting in plants from the cross triploid × glomerata.*

Chromosome number	Number of seeds per 1 gr. of panicle substance: 0	250	500	750	1000	1250	1500	1750	2000	2250	2500	2750	3000	3250	3500	3750	4000	4250	n	M ± m
22 + 23		1																	1	125 ± 610
24 + 25		6	5	2															13	298 ± 169
26 + 27		17	11	18	2	6	2	—	2	—	—	—	—	—	—	—	—	1	59	620 ± 80
28		6	4	2	4	3	2	—	—	1	—	1	—	—	—	—	—	1	24	917 ± 125
29 + 30		6	2	1	1	2													12	437 ± 176
31 + 32			1	1	—		1												3	792 ± 353
33 + 34		2	1	4	1	2													10	625 ± 193
35 + 36		3	4	4	5	2													18	610 ± 144

B. SEED SETTING.

As in *D. glomerata*, the highest seed production was met with in the 28-chromosome class, the average value of this class being 917 ± 125 in the present material (Table 16). The number of individuals examined is rather limited, but if all the other variates are added into one series the average of this series will be 494 ± 57. The difference between this value and that of the 28-chromosome class is 423 ± 138 and is consequently significant (D/m = 3,07).

7. PROGENIES OF PENTAPLOIDS.

So far pollen fertility has been examined in only 74 daughter plants of pentaploids. Three of these plants, having the chromosome numbers 32, 33 and 35, proved to be male sterile, in the rest of the material pollen was produced, the percentage of good pollen ranging from 30 to 100 per cent. The following average values were obtained:

Chromosome number:	28	29	30	31	32	33	34	35	36	37	38	39
Average value:	95,0	95,0	83,0	85,0	75,8	65,0	79,7	82,5	81,7	95,0	95,0	75,0
Number of individuals:	1	1	5	5	13	5	15	12	9	1	3	1

The only safe conclusion that can be drawn from these values is that the average pollen fertility in all the classes is rather high. The values intermediate between 28 and 35 seem to represent a slight decrease in pollen fertility, precisely as in vigour (cf. Table 11, p. 173), but the differences are based on too few values to be significant.

A preliminary study of seed production after open pollination was also undertaken in this material. However, only 34 individuals were examined, and no differences between the different chromosome classes could be detected. The average value of all the plants was found to be as low as 287.

8. COMBINATION OF ALL DATA ON POLLEN FERTILITY.

As was done in the case of vigour, it seemed desirable to sum up all the data on pollen fertility into a single series. The result of this summation is given in Table 17 and Diagram 2. With regard to the chromosome numbers 14—21 the construction of the curve was very simple. The pollen fertility of the 14-class corresponds to the total average value of *D. Aschersoniana*, which was found to be 84,0. As all the plants in the classes 15 to 21 were completely male sterile with non-dehiscing anthers the curve in this interval is drawn at the value 0. It is true that the closed anthers (at least in the triploids) contained

a low percentage of apparently good pollen, but in any case *there is a very deep minimum between 14 and 22.*

The average values, corresponding to the chromosome numbers 22 to 41, were obtained by summation of all values from pure and aberrant *glomerata*, from triploid × *glomerata* and from the progenies of pentaploids. The resulting series and average values are represented in Table 17. As is at once evident from the means and standard errors

TABLE 17. *Correlation between chromosome number and pollen fertility. Total of all values available.*

Chromosome number	Male sterile	Per cent male sterile	Percentage of good pollen									n	M ± m
			20	30	40	50	60	70	80	90	100		
22	1	33		1	1							2	30,0 ± 9,8
24	1	100										—	—
25	4	27			1	—	—	5	4	—	1	11	68,6 ± 4,2
26	6	14		1	3	1	7	4	6	8	6	36	70,0 ± 2,3
27	8	8			1	—	16	15	18	15	26	91	76,8 ± 1,5
28	8	4			2	1	5	12	21	37	100	178	86,5 ± 1,0
29	8	12				1	6	7	20	9	15	58	77,9 ± 1,8
30	2	5				1	1	8	4	9	14	37	81,5 ± 2,3
31	1	8						1	2	6	2	11	83,2 ± 4,2
32	0	0					2	2	5	4	3	16	77,5 ± 3,5
33	4	29			1	1	—	2	—	2	4	10	76,0 ± 4,4
34	0	0					1	3	4	4	10	22	83,6 ± 3,0
35	0	0						3	—	8	12	23	87,6 ± 2,9
36	1	5						2	2	5	9	18	86,7 ± 3,3
37	0	0								1	1	2	90,0 ± 9,8
38	0	0									3	3	95,0 ± 8,0
39	0	0							1			1	75,0 ± 13,9
41	0	0								1		1	85,0 ± 13,9

there is a marked and significant increase of pollen fertility as the chromosome numbers increase from 22 to 28. Above 28 the differences are less clear, but a closer examination of the data shows that *there is a minimum between 28 and 35.* If the classes are added three and three the average value of the classes 28 + 29 + 30 will be 84,0 ± 0,8, the corresponding values of the classes 31 + 32 + 33 and 34 + 35 + 36 being 78,8 ± 2,3 and 86,0 ± 1,8 respectively. The difference 84,0 — 78,8 is 5,2 ± 2,4 (D/m = 2,17) and the difference 86,0 — 78,8 is equal to 7,2 ± 2,9 (D/m = 2,48). The occurrence of the fertility minimum in question is also evident, if the average values of the euploid classes

28 and 35 are compared with the total average of the aneuploid classes. The latter value, based on the 29 + 30 + 31 + 32 + 33 + 34 classes, was found to be 79,8 ± 1,1, the average values of the 28- and 35-classes being 86,5 ± 1,0 and 87,6 ± 2,9. The difference 86,5 — 79,8 is 6,7 ± 1,5 (D/m = 4,47), and 87,6 — 79,8 is equal to 7,8 ± 3,1 (D/m = 2,52). Thus, it is quite certain that there is a decrease in fertility on both sides of

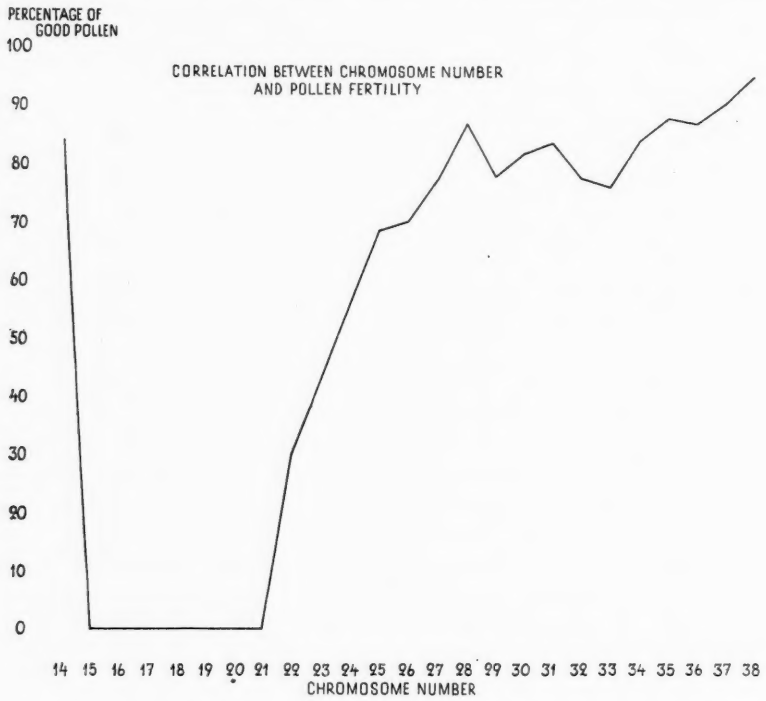

Diagram 2.

the maximum at 28. It is also extremely probable that this decrease is followed by a new increase near the pentaploid number. The apparent maximum at 31 is not significant.

The percentage of male sterile plants, also given in Table 17, is evidently highest in the classes with low chromosome numbers. The average percentage in the entire material is 7,80, 44 male sterile being found in a total of 564 plants. The average percentage of the 28-class is 4,30 ± 1,97, the corresponding value of all classes below 28 being

$12{,}_{50} \pm 2{,}_{12}$ (20 male steriles among 160 plants). The difference between these values is $8{,}_{20} \pm 2{,}_{89}$ ($D/m = 2{,}_{84}$) and may thus be regarded to be significant. The average value of male steriles among the classes above 28 is $7{,}_{34}$, a value not significantly higher than that of the 28-chromosome class.

V. CHROMOSOME NUMBER AND MORPHOLOGY.

1. INTRODUCTION.

The morphology of the present material has been studied from two reasons: firstly, in order to investigate the general correlation between chromosome number and different morphological characters, secondly and especially, in order to elucidate, as far as possible, the real nature of the species difference *Aschersoniana—glomerata*. On account of the great chromosomal variation good opportunities are afforded for studying the morphological changes brought about by different chromosome numbers, and at the same time this variation is of immediate interest for an understanding of the fact that the spontaneous *Dactylis* types occur as two different species.

Of these species *D. Aschersoniana* was distinguished taxonomically much later than *glomerata* and was originally considered to be a variety of *glomerata* (var. *lobata* DREJ.). It was first described as a distinct species by GRÆBNER (cf. HOLMBERG, 1926) as late as 1899. TURESSON (1922, 1929 a) regards *Aschersoniana* as a shade ecotype of *glomerata*. SAMUELSSON (1923) has given a review of the taxonomical treatment of the unit in question, and on the basis of a study of Scandinavian material he concludes that *Aschersoniana* should at least have the rank of a subspecies. In the Scandinavian Flora edited by HOLMBERG (1926), finally, *Aschersoniana* is regarded as a separate species of the same rank as *glomerata*.

As is evident from the diagnoses given in the floristical textbooks the morphological species differences between *Aschersoniana* and *glomerata* are rather vague, and as SAMUELSSON says (SAMUELSSON, 1923, pp. 134—135) none of the special characters taken one by one is enough to distinguish the two types, though the total combination spheres of characters are clearly different.

In order to get a starting point for a comparative morphological analysis of the present *Dactylis* material the following seven characteristic differences between *glomerata* and *Aschersoniana* were chosen with the aid of the descriptions given in HOLMBERG's Flora:

Morphological difference No.	*D. glomerata*	*D. Aschersoniana*
1. Mode of growth:	Densely tufted, culms tall and thick	More loosely tufted, culms shorter and thinner
2. Leaf colour ...:	Greyish or bluish green	Vividly light green-yellowish green
3. Sheaths .....:	Scabrid	Smooth
4. Panicles .....:	Stiff and erect, spikelets in glomerate clusters. Basal panicle branches coarse and stiff	Somewhat decumbent, the apex narrow and elongated. Basal panicle branches thin as threads and rather slack. Clusters of spikelets lax
5. Number of flowers per spikelet:	4—5 flowers	5—6 flowers
6. Glumes .....:	With stiff hairs	Smooth
7. Shape of flower glumes .....:	Flower glumes in the upper part of the spikelet tapering with a long acumen	Flower glumes in the upper part of the spikelet stunted or incised with a short acumen

Typical spikelets of *Aschersoniana* and *glomerata* are represented in Fig. 73. The differences in hairiness, number of flowers and length of the acumens are plainly visible. — In the beginning of the work an eighth character was also studied, *viz.* the clear presence or absence of nerves on the bracts. It was soon found out that this character as well as the size of the spikelets was useless for classification, and hence the morphological studies were entirely based on the seven differences described above.

With respect to each of these characters the plants might be of *glomerata*- or *Aschersoniana*-type or intermediate, these three possibilities being marked by +, — or 0 respectively. On the basis of these signs the total morphological values of the plants were calculated, a plus being counted as + 1, a minus as — 1 and a nought as ± 0. Accordingly, a plant which was *glomerata*-like in all the seven characters would be given the value + 7 and, inversely, the most typical

Aschersoniana individuals would be — 7. A plant of the type + + + — 0 — 0, for instance, would be given the value + 1. The same value may of course represent quite different morphological constellations

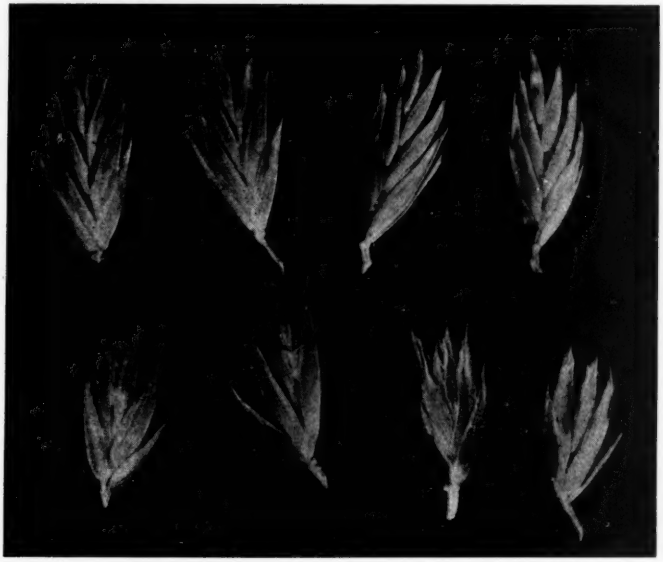

Fig. 73. Typical spikelets of *Dactylis Aschersoniana* (upper row) and *D. glomerata* (below). — Magnification: 4 ×.

(e. g. — — — + + + +), and the morphological values merely denote the average proportion of *glomerata* and *Aschersoniana* characters. As such, however, they proved to be rather valuable.

2. MORPHOLOGY OF THE TWO SPECIES AND SPONTANEOUS HYBRIDS.

The morphological values of the two species, *Aschersoniana* and *glomerata*, and of some spontaneous triploid hybrids are given in Table 18. The values of typical *glomerata* and *Aschersoniana* do not overlap at all, the average values being + 5,00 and — 4,92 respectively. The triploids are intermediate with a prevalence of the *glomerata* characters, the average value being + 2,58.

Though the morphological values of the two species are quite distinct there is a good deal of intra-specific variation, the values in *D. Aschersoniana* ranging from — 7 to — 3 and those of *glomerata*

from + 3 to + 7. In part this variation may be caused by genetical differences. The mother clone of the commercial variety Skandia II, for instance, was studied morphologically in two different years. In the first year this resulted in the average value + 3, in the second year the average value was found to be + 5. A progeny after isolation of Skandia II has been raised, and in 19 individuals, controlled to have 2n = 28, the morphological values ranged from + 1 to + 7 and were on an average + 4,47. The morphology of the mother clone and the average value of the progeny obviously correspond to each other rather well. Other biotypes of *D. glomerata* may be more extreme and typical. For instance, the mother clone of the commercial variety »1098» was examined in three different years, the values + 6, + 5 and + 7 being obtained.

At the same time as the typical representatives of the two species, a number of plants were studied which had either 14 or 28 chromosomes and which had been obtained from the same hybrid populations in

TABLE 18. *Morphology of Dactylis glomerata, Aschersoniana and hybrids between these species.*

Species or hybrids	Chromosome number	Morphological value: −7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	n	M ± m
D. glomerata, Single individuals from different varieties	28										2	2	2	4	1		11	+ 5,00 ± 0,59
D. glomerata. Individuals from hybrid populations ...	»						1	—	—	1	2	4	4	5			17	+ 3,29 ± 0,40
glomerata × *Aschersoniana* (spontaneous hybrids)	21							1	1	—	1	7	1	1			12	+ 2,58
D. Aschersoniana, from hybrid populations.....	14	3	1	3	1	—	1										9	— 5,33
D. Aschersoniana	»	1	2	6	3	1											13	— 4,92
D. Aschersoniana, total	»	4	3	9	4	1	1										22	— 5,09

the neighbourhood of Svalöf as the triploid hybrids. As regards the plants with 14 chromosomes, belonging to this category, their average value (— 5,33) was not significantly different from that of the other *Aschersoniana* types [1] (— 4,92) and consequently these plants show no trace of *glomerata* influence. On the other hand, the plants with 28 chromosomes, obtained from hybrid populations, have a lower average value than the typical *glomerata* plants, the morphological values being + 3,29 ± 0,40 and + 5,00 ± 0,50 respectively. The difference is 1,71 ± 0,64 (D/m = 2,67), the odds that this difference is significant being 132 to 1. As the typical *glomerata* plants all belong to the rather representative material cited in Table 2, the conclusion must be drawn that *the 28-chromosome plants from hybrid populations are somewhat similar to Aschersoniana on account of previous hybridisation with this species.* The cause of this morphological change will be further discussed below (p. 208).

3. THE TRIPLOID × GLOMERATA MATERIAL.

The seven morphological species differences were also studied in 215 plants, obtained from the crosses triploid × *glomerata*. The result of this work is summarized in Table 19. The total average value is + 2,86 ± 0,13 and is thus intermediate between the value found to be characteristic of the triploid hybrids (+ 2,58) and *glomerata* (+ 5,00). A somewhat higher figure might have been expected, but the rather low value obtained is probably due to the fact that the *glomerata* type almost exclusively used for the crosses, Skandia II, is not a very extreme *glomerata*, its morphological value, as mentioned above, being only about + 4. — In this connection it should be mentioned that the few plants obtained from the cross triploid × *Aschersoniana* were, on the average, intermediate between the triploids and *Aschersoniana*, the average value of eight plants being + 0,63.

Recurring to Table 19, we see at once that there is a most obvious correlation between chromosome number and morphology, the average values of the lower classes ranging from + 1 to + 3 and of the higher classes from + 3 to + 7. Consequently, *as the chromosome number increases the glomerata characters become more and more pronounced.* The differences between the individual classes are not significant, but if the values are divided into three groups: those obtained from plants with exactly 28 chromosomes and those from lower or higher chromosome numbers, the three series represented in the lower part of Table 19

[1] Most of this material is derived from plants collected at Kalundborg, Denmark.

are obtained. The average values of these series are $+2{,}21 \pm 0{,}18$ (lower than 28), $+2{,}87 \pm 0{,}31$ (28) and $+4{,}25 \pm 0{,}26$ (higher than 28). The difference $4{,}25 - 2{,}21$ is equal to $2{,}04 \pm 0{,}32$ ($D/m = 6{,}38$) and the difference $4{,}25 - 2{,}87$ is $1{,}38 \pm 0{,}40$ ($D/m = 3{,}45$). Thus, the plants with higher chromosome numbers than 28 are significantly different from those with 28 chromosomes or lower numbers.

TABLE 19. *Triploid × glomerata. Correlation between chromosome number and morphology.*

Chromosome number	Morphological value: −3	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	n	M ± m
22						3						3	+2,00 ± 1,10
24					1							1	+1,00 ± 1,91
25			1	2	2	1	6	2	—	2		16	+2,56 ± 0,48
26	2	3	4	4	3	6	8	4	2	1		37	+1,49 ± 0,31
27			4	4	7	12	17	14	3	1	1	63	+2,57 ± 0,24
28		1	1	3	5	7	7	5	5	5		39	+2,87 ± 0,31
29						3	4	2	1	1		11	+3,36 ± 0,58
30					1	2	—	1				4	+2,25 ± 0,96
31					1	—	—	2				3	+3,00 ± 1,10
32							1	—	1			2	+4,00 ± 1,35
33					1	—	2	2	1	—	1	7	+3,86 ± 0,72
34								2	1	4		7	+5,29 ± 0,72
35					1	—	—	4	2	2	3	12	+5,00 ± 0,55
36				1	—	—	—	1	2	3	1	8	+4,88 ± 0,68
37									1			1	+5,00 ± 1,91
41											1	1	+7,00 ± 1,91
Σ lower than 28	2	3	9	10	13	22	31	20	5	4	1	120	+2,21 ± 0,18
28		1	1	3	5	7	7	5	5	5		39	+2,87 ± 0,31
Σ higher than 28				1	4	5	7	14	9	10	6	56	+4,25 ± 0,26
Total	2	4	10	14	22	34	45	39	19	19	7	215	+2,86 ± 0,13

The observed correlation between high chromosome numbers and *glomerata* morphology might be due, either to a higher frequency of specific *glomerata* genes or to the high chromosome numbers as such. At the same time this question involves the important problem, *whether the species difference glomerata—Aschersoniana is mainly caused by gene differences or mainly by the difference in chromosome number.*

This is, in fact, one of the main problems dealt with in the present paper.

Assuming the first alternative, that the genomes of *glomerata* and *Aschersoniana* are rather different genetically, the triploid hybrids will contain seven *Aschersoniana* (A-) *chromosomes* and fourteen *glomerata* (G-) chromosomes. From the mode of meiosis in the triploids we know that in each of the seven sets of three chromosomes distribution must be at random, two chromosomes generally passing to one pole and one chromosome to the other pole. There is no reason to believe that the *Aschersoniana* chromosomes have a fixed position in the trivalents, and if this is not the case the average proportion of G- and A-chromosomes in the gametes will be the same as in the somatic cells. Consequently, the gametes formed by the triploids must on an average contain twice as many *glomerata* as *Aschersoniana* chromosomes, and this proportion is in all probability independent of the chromosome elimination occurring.

If the morphology of the plants depends on the relative proportion of A and G genes, the plants with high chromosome numbers and *glomerata* morphology, obtained from the cross triploid $\times$ *glomerata*, should contain a relatively higher number of G-genes than the other category, plants with lower numbers and a tendency to be *Aschersoniana*-like. However, a consideration of the relative proportion of G- and A-chromosomes in plants with high and low chromosome numbers gives the following result: All male gametes (from pure *glomerata*) contain ($\pm$) 14 G-chromosomes. If such a gamete meets a reduced ovule, e. g. with 12 chromosomes, the resulting plant will have $2n = 26$. On an average the 12 chromosomes of the female gamete will comprise 8 *glomerata* and 4 *Aschersoniana* chromosomes, hence the ratio of G- and A-chromosomes in the fertilized ovule will be $(14 + 8) : 4 = 5{,}5$. On the other hand, in a pentaploid, arisen from the union of an unreduced ovule and a reduced male gamete, the corresponding ratio will be $(14 + 14) : 7 = 4{,}0$. Thus in the pentaploids there must be a higher proportion of A-genes than in the plants with 26 chromosomes. This conclusion may immediately be extended to the assertion that on the plausible assumptions made there must be a positive correlation between chromosome number and proportion of A-chromosomes. — As may easily be controlled the ratio of G : A chromosomes in the different chromosome classes of Table 19 should fall according to the following series (chromosome classes in brackets): $7{,}2$ (22), $6{,}2$ (24), $5{,}8$ (25),

5,5 (26), 5,2 (27), 5,0 (28), 4,8 (29), 4,6 (30), 4,5 (31), 4,3 (32), 4,2 (33), 4,1 (34), 4,0 (35), 3,9 (36), 3,8 (37), and 3,6 (41).

If the morphology of the material under discussion were gene controlled in the way assumed, there should be a *negative* correlation between chromosome number and *glomerata* morphology, and the plants similar to *Aschersoniana* should be found among the higher chromosome classes. Inasmuch as quite the opposite result was obtained, the conclusion must be drawn that in the main the morphological species differences between *glomerata* and *Aschersoniana* are not caused by specific genes, present in one of the species and absent in the other.

Before leaving the hypothesis of genic differences between the two species, another possibility may be considered and rejected, viz. that *Aschersoniana* has the constitution AA and *glomerata* AABB. — The mode of meiosis in *glomerata* and the triploid hybrids shows that the presumed A- and B-genomes are cytologically homologous, but nevertheless there might be a genic differentation, one of the genomes being homologous to the *Aschersoniana* genome, the other being responsible for the morphological characteristics of *glomerata.* However, in this case too there should be a negative correlation between chromosome number and *glomerata* habitus in the triploid $\times$ *glomerata* material. This is evident from the same examples as those used above, involving the constitution of a plant with 26 chromosomes, arisen from a reduced ovule, and a plant with 35 chromosomes arisen from an unreduced female gamete. In the first case the ovule will contain 8 A- and 4 G-chromosomes and the male gamete 7 A- and 7 G-chromosomes. This gives the ratio 11 G : 15 A = 0,73. In the second case the ovule will contribute 14 A- and 7 G-chromosomes the male gamete, as before, 7 A + 7 G. The total ratio will be 14 G : 21 A = 0,67. Taking the extreme chromosome classes (22 and 41) of Table 19, the corresponding values of these classes would be 0,78 and 0,64 respectively.

It is obvious that the results actually obtained are not at all in accordance with the hypothesis discussed above, that the two species have the constitutions AA and AABB. As there does not seem to remain any other hypothesis of a similar type, it is necessary to conclude that *the morphological species differences between Dactylis Aschersoniana and glomerata are caused, at least to the greatest extent, by the quantitative difference in chromosome number and not by specific genes present in one of the species and absent in the other.*

This conclusion is strongly supported also by the following data.

4. PROGENIES OF PENTAPLOIDS.

Only 44 daughter plants of four different pentaploids have been studied so far with regard to the morphological details. The chromosome numbers of these plants varied between 28 and 39 and were on the average 33,4. The morphological average values in the different classes were found to be the following:

Chromosome number:	28	29	30	31	32	33	34	35	36	37	38	39
Average morphology:	6,0	7,0	4,4	4,0	4,0	4,3	4,7	5,5	4,5	5,0	5,0	5,0
Number of individuals:	1	1	5	2	9	3	7	7	4	3	1	1

There are no significant differences between the classes, but an interesting point is that all the values represent a rather marked *glomerata* morphology. The total average of the entire material was found to be $+ 4{,}75 \pm 0{,}24$. The morphological values of the mother individuals were slightly higher ($+ 5$, $+ 6$, $+ 6$, $+ 7$). It should be remembered, however, that the chromosome numbers of the mother plants (34, 35, 36 and 36) were also higher than the average chromosome number of the progenies (33,4). — The high morphological values of the progenies of pentaploids furnish additional evidence that high chromosome numbers in *Dactylis* are correlated with *glomerata* habitus.

5. PROGENIES OF PURE AND ABERRANT GLOMERATA.

If the results obtained above are correct, viz. that the species differences are not altogether caused by specific *Aschersoniana* or *glomerata* genes, the chromosome variation in *D. glomerata* should also influence the same morphological characters. As is evident from Table 20 this was really found to be the case. As the chromosome number increases from 26 to 32, the morphological values increase from $+ 1$ to $+ 7$, or, if the extreme and unreliable values are omitted, the change in chromosome number from 27 to 30 is accompanied by an increase of the morphological average from $+ 4{,}04$ to $+ 5{,}15$. The differences between the separate classes are not significant, but if the materials is divided into the three series represented in the lower part of Table 20, the following values are obtained. Plants with lower numbers than 28 have the average value of $+ 3{,}81 \pm 0{,}38$, the corresponding values of those with exactly 28 and higher numbers than 28 being $+ 4{,}19 \pm 0{,}25$ and $+ 5{,}00 \pm 0{,}28$ respectively. The difference $5{,}00 - 3{,}81$ is $1{,}19 \pm 0{,}47$ and $D/m = 2{,}53$. The odds that this difference is significant are 88 to 1.

Thus, with a high degree of probability it can be stated that

glomerata aberrants with lower numbers than 28 have a tendency to become Aschersoniana-like, and, on the other hand, the aberrants with higher numbers than 28 are super-glomerata types with the species characters of *glomerata* somewhat exaggerated (as compared with the 28-chromosome class). *This result definitely shows that purely quantitative changes influence the morphological species characters.* In this

TABLE 20. *Dactylis glomerata. Correlation between chromosome number and morphology.*

Chromosome number	Morphological value:										n	M ± m
	−2	−1	0	+1	+2	+3	+4	+5	+6	+7		
26		1	—	—	—	1					2	+1,00 ± 1,36
27			2	1	2	4	4	4	5	2	24	+4,04 ± 0,39
28	1	1	—	2	6	9	12	11	13	4	59	+4,19 ± 0,25
29					3	3	1	4	8	2	21	+4,81 ± 0,42
30			1	—	1	2	2	3	5	6	20	+5,15 ± 0,43
31				1	—	—	—	1	1	1	4	+4,75 ± 0,96
32										1	1	+7,00 ± 1,93
Σ lower than 28		1	2	1	2	5	4	4	5	2	26	+3,81 ± 0,38
28	1	1	—	2	6	9	12	11	13	4	59	+4,19 ± 0,25
Σ higher than 28			1	1	4	5	3	8	14	10	46	+5,00 ± 0,28
Total	1	2	3	4	12	19	19	23	32	16	131	+4,40 ± 0,17

case there cannot be any question of the relative frequency of *glomerata* and *Aschersoniana* genes, since the material is exclusively procured by intra-specific variation in *glomerata* and has nothing to do with the other species. Nevertheless, the aberrants with low numbers are similar to *Aschersoniana* and those with high numbers are super-*glomerata* types. This result is in very close agreement with the conclusions drawn from the triploid × *glomerata* material.

6. A DETAILED ANALYSIS OF THE SEVEN SPECIES DIFFERENCES.

Although it has been demonstrated above that there is a total effect of quantitative chromosome changes on the morphological species characters, it must be admitted that this effect may be caused mainly by one or a few characters, though the other species differences are gene controlled and independent of the chromosome number.

TABLE 21. *Triploid × glomerata. Correlation between chromosome number and seven morphological species differences.*

Morphological character number	*Aschersoniana* (—), *glomerata* (+) or intermediate type (0)	Chromosome number: 22...24	25	26	27	28	29	30	31	32	33	34	35	36	37...41	n	M ± m	Difference between + and — average
1	—	2 —	3	8	5	1	1	—	1	—	1	—	1			23	$26{,}87 \pm 0{,}67$	
»	0	1 1	2	12	22	13	3	1	2	1	2	—	—	1		61	$27{,}52 \pm 0{,}41$	$+2{,}13 \pm 0{,}72$
»	+		11	17	36	25	7	3	—	1	4	7	11	7	1 1	131	$29{,}00 \pm 0{,}28$	(D/m = 2,96)
2	—	1 1	1	6	4	3	1									17	$26{,}35 \pm 0{,}79$	
»	0		1	8	16	11	1	3	—	—	2	1	1			44	$27{,}89 \pm 0{,}49$	$+2{,}36 \pm 0{,}83$
»	+	2 —	14	23	43	25	9	1	3	2	5	6	11	8	1 1	154	$28{,}71 \pm 0{,}26$	(D/m = 2,84)
3	—	1 1	1	12	15	7	5	—	1	—	5	2	3	1	1	55	$28{,}56$	
»	0	2 —	1	5	13	12	3	3	—	—	1	1	1			42	$27{,}74$	$-0{,}11$
»	+		14	20	35	20	3	1	2	2	1	4	8	7	— 1	118	$28{,}45$	
4	—	2 —	3	9	8	6										28	$26{,}32 \pm 0{,}59$	
»	0		4	12	17	13	5	2	1	1	1	—	1			57	$27{,}56 \pm 0{,}42$	$+2{,}82 \pm 0{,}65$
»	+	1 1	9	16	38	20	6	2	2	1	6	7	11	8	1 1	130	$29{,}14 \pm 0{,}28$	(D/m = 4,34)
5	—		6	6	17	5	3	1	—	—	—	—	—	1		39	$27{,}13 \pm 0{,}52$	
»	0	2 —	4	18	21	11	2	2	2	1	2	2	7	6		80	$28{,}68 \pm 0{,}36$	$+1{,}45 \pm 0{,}62$
»	+	1 1	6	13	25	23	6	1	1	1	5	5	5	1	1 1	96	$28{,}58 \pm 0{,}33$	(D/m = 2,34)
6	—		8	21	27	19	—	—	1	—	—	1	1	1		79	$27{,}13 \pm 0{,}36$	
»	0		2	3	9	3	3	2	—	1	2	2	1			28	$28{,}68 \pm 0{,}60$	$+2{,}04 \pm 0{,}47$
»	+	3 1	6	13	27	17	8	2	2	1	5	4	10	7	1 1	108	$29{,}17 \pm 0{,}30$	(D/m = 4,34)
7	—		5	4	3	2	—	—	—	1	—	—	—	1		16	$27{,}12 \pm 0{,}81$	
»	0	2 1	3	14	23	12	3	4	1	—	—	1	3	2		69	$27{,}72 \pm 0{,}39$	$+1{,}72 \pm 0{,}86$
»	+	1 —	8	19	37	25	8	—	2	1	7	6	9	5	1 1	130	$28{,}84 \pm 0{,}29$	(D/m = 2,00)

In order to elucidate this question it was necessary to study the correlation between chromosome number and each of the seven morphological species differences separately. This was done by calculating the average chromosome numbers of plants with +, — or 0 values (Tables 21—22) and by comparing the means thus obtained. If there is no difference between the three values, the morphological character in question is independent of the chromosome numbers, and in the opposite case the correlation may either be positive or negative. Since a positive correlation had already been demonstrated with respect to the *total* of the seven differences, at least one or a few of the individual differences must show a similar correlation.

In *the triploid* × *glomerata material* the following conditions were met with (Table 21). In regard to six of the seven differences the average chromosome number of the + individuals was higher than that of the — individuals. Thus the total correlation observed is the expression of a similar correlation in the majority of the species differences. Only difference No. 3 (smooth or scabrid sheaths) forms a marked exception and seems to be quite independent of the chromosome number, the average values being $28{,}56$ (—), $27{,}74$ (0) and $28{,}45$ (+). In regard to five of the other six morphological differences the — plants had the lowest average chromosome number, the 0 plants were intermediate and the + plants had the highest values. In the case of difference No. 5 (number of flowers per spikelet) the + plants had a higher average value ($28{,}58 \pm 0{,}33$) than the — plants ($27{,}13 \pm 0{,}52$) but the value of the 0 plants was still higher ($28{,}68 \pm 0{,}36$). The difference between these + and 0 values, however, is not significant, and, on the other hand, the odds that the difference between the + and — values is significant are as high as 52 to 1, D/m being $2{,}34$. The five other morphological differences show a quite clear correlation between high chromosome number and similarity to *glomerata*, the numerical differences between the + and — values being $2{,}13 \pm 0{,}72$ ($D/m = 2{,}96$), $2{,}36 \pm 0{,}83$ ($D/m = 2{,}84$), $2{,}82 \pm 0{,}65$ ($D/m = 4{,}34$), $2{,}04 \pm 0{,}47$ ($D/m = 4{,}34$) and $1{,}72 \pm 0{,}86$ ($D/m = 2{,}00$) [1]. Of these differences only the last-mentioned one is doubtful, the odds of its significance being 22 to 1.

The greatest numerical differences between the + and — variants are shown by characters No. 4 (type of panicle), No. 2 (leaf colour)

[1] The standard errors are based on the total Σd^2 of the three series compared. In some cases the series are bimodal but, nevertheless, it may be correct to calculate the standard errors and compare the average values, since the differences between the average values of the series will show an approximately normal distribution.

and No. 1 (mode of growth) but also with respect to character No. 6 (hairy or smooth glumes) there is a marked and significant difference. (The high D/m value in this case is mainly caused by the unusually large number of individuals in the minus class).

TABLE 22. *Dactylis glomerata. Correlation between chromosome number and seven morphological species differences.*

Morphological character number	*Aschersoniana* (—), *glomerata* (+) or intermediate type(0)	Chromosome number: 26	27	28	29	30	31	32	n	M ± m	Difference
1	— and 0	1	8	24	3	3	1		40	$28{,}05 \pm 0{,}18$	$+ 0{,}47 \pm 0{,}21$
»	+	1	16	35	18	17	3	1	91	$28{,}52 \pm 0{,}12$	(D/m = 2,24)
2	— and 0	2	5	10	2	6	1		26	28,31	+ 0,08
»	+		19	49	19	14	3	1	105	28,39	
3	— and 0		3	9	3	5	1		21	28,62	— 0,29
»	+	2	21	50	18	15	3	1	110	28,33	
4	— and 0	1	8	31	4	2	1		47	$28{,}02 \pm 0{,}16$	$+ 0{,}55 \pm 0{,}20$
»	+	1	16	28	17	18	3	1	84	$28{,}57 \pm 0{,}12$	(D/m = 2,75)
5	— and 0	2	12	25	15	6	2		62	28,27	+ 0,19
»	+		12	34	6	14	2	1	69	28,46	
6	— and 0	1	12	25	6	6			50	$28{,}08 \pm 0{,}16$	$+ 0{,}48 \pm 0{,}20$
»	+	1	12	34	15	14	4	1	81	$28{,}56 \pm 0{,}12$	(D/m = 2,40)
7	— and 0	1	3	9	2				15	$27{,}80 \pm 0{,}29$	$+ 0{,}65 \pm 0{,}31$
»	+	1	21	50	19	20	4	1	116	$28{,}45 \pm 0{,}10$	(D/m = 2,10)

An analysis of the same type as described above was also undertaken in *the material of D. glomerata* available. The results of this investigtion are summarized in Table 22. As the number of individuals was rather low in the minus classes, it was necessary to combine the variates of the — and 0 classes into one series. The average of this series, however, may be compared with that of the corresponding + series. — Two facts are at once evident from Table 22. Firstly, there is no correlation between chromosome number and character No. 3 (scabrid or smooth sheaths), the difference between the two classes being — 0,29. Secondly, one of the greatest differences observed ($+ 0{,}55 \pm 0{,}20$) concerns character No. 4 (type of panicle). Exactly the same result was reported above with regard to the triploid × *glomerata* material. This may be considered to prove that the first morphological difference

in question (No. 3) is controlled by specific genes, the other difference (No. 4) more directly by the chromosome number.

As regards the other characters the + variants in Table 22 have in all cases the highest average value, but the differences are not great enough to be significant. In the case of characters 1, 6 and 7, however, the D/m values range from 2,10 to 2,40, making it highly probable that these characters are really correlated with chromosome number. As described above, this was also the case in the triploid × *glomerata* material (Table 21).

Conclusion. — Most of the seven species differences studied are influenced by changes in chromosome number, but the degree of this correlation is different for different characters. One morphological species character (smooth or scabrid sheaths) *was even found to be quite independent of the chromosome number.*

7. CORRELATION BETWEEN CHROMOSOME NUMBER AND CULM DIAMETER.

The morphological species difference No. 1 is really a complex one, involving the mode of growth as well as the length and thickness of the culms. During the morphological studies I obtained the impression that the thickness of the culms was rather strongly correlated with the chromosome number and this impression was, indeed, verified by direct measurements. The data in Table 23 show the diameter of the culms (just below the panicle) in various *Dactylis* types with different chromosome numbers. In *D. glomerata* the average culm diameter was found to increase quite continuously from 12,00 (2n = 26) to 17,00 (2n = 32). — Instead of calculating the standard errors in the usual way I found it convenient in the present case (the *glomerata* classes) to calculate the coefficient of correlation, the r value obtained being + *0*,197. The odds that this coefficient is significant are about 1000 to 1.

The average value of the 28-chromosome class, 14,82, may be compared with the mean values of *Aschersoniana* (11,60) and the triploid hybrids (14,40). Though the number of variates in the latter two series is rather low, the occurrence of a significant positive correlation between chromosome number and culm diameter cannot be doubted.

The occurrence of such a correlation could be definitely demonstrated also in the case of the triploid × *glomerata* material. The results of these measurements are given in the lower part of Table 23. The average value of plants with exactly 28 chromosomes was found

to be $15{,}34 \pm 0{,}36$, the corresponding figures of plants with lower and higher chromosome numbers being $14{,}48 \pm 0{,}21$ and $16{,}52 \pm 0{,}31$. The difference $16{,}52 - 14{,}48$ is $2{,}04 \pm 0{,}38$ ($D/m = 5{,}37$) and is consequently quite significant. The other differences are less reliable, $16{,}52 - 15{,}34$ being equal to $1{,}18 \pm 0{,}48$ ($D/m = 2{,}46$) and $15{,}34 - 14{,}48 = 0{,}86 \pm 0{,}42$ ($D/m = 2{,}05$).

TABLE 23. *Correlation between chromosome number and culm diameter in various Dactylis types.*

Species or cross combination	Chromosome number	Culm diameter in tenths of 1 mm. 6	8	10	12	14	16	18	20	22	24	n	M ± m
D. Aschersoniana	14		1	5	4							10	11,60
D. glomerata × *Asch.* F_1	21				4	5	1					10	14,40
D. glomerata (progenies from pure and aberrant individuals)	26			1	1							2	12,00
	27			6	8	15	1					30	13,74
	28	2	4	16	27	58	27	11	4	1		150	14,82
	29			3	13	17	10	5	—	1		49	15,20
	30		1	2	4	7	13	2	1			30	15,60
	31			1	—	—	1	1				3	15,66
	32						1					1	17,00
Triploid hybrids × *glomerata*	lower than 28		3	14	31	45	22	4	2			121	14,48 ± 0,21
	28			2	8	16	12	2	1			41	15,34 ± 0,36
	higher than 28			1	7	17	14	11	5			55	16,52 ± 0,31

Although the three series just mentioned are the only ones published it may be of interest to mention the average values of each chromosome class of the triploid × *glomerata* material. The following values were found (the chromosome numbers in brackets): 12,3 (22), 13,0 (24), 13,8 (25), 13,3 (26), 14,7 (27), 14,8 (28), 15,2 (29), 15,5 (30), 15,3 (31), 16,5 (32), 16,0 (33), 16,6 (34), 15,8 (35), 17,7 (36), and 20,0 (41). In spite of the fact that several of these values are based on only one or a few variates the positive correlation between culm diameter and chromosome number is very pronounced.

8. CORRELATION BETWEEN CHROMOSOME NUMBER AND CELL SIZE.

As many of the morphological differences discussed above may be the expression of a changed cell size, measurements of pollen grains and stomata were undertaken in a series of *Dactylis* types with differ-

ent chromosome numbers. The data obtained are given in Tables 24—25.

A. POLLEN DIAMETER.

Many of the values in Table 24 represent the total average of several series of measurements which have been combined into one value. This was done by using the pollen of pure *glomerata* as a standard, to which was given the value 100. Therefore the figures

TABLE 24. *Correlation between chromosome number and pollen size.*

Chromosome number	Average pollen diameter	Number of tests	Number of plants examined	Total number of pollen grains measured
14	85,7	10	8	540
15	78,0	1	1	50
16	74,0	1	1	50
17	88,0	1	1	50
19	75,0	1	1	50
20	78,0	1	1	50
21	85,8	5	5	400
22	82,0	1	1	50
24	86,0	1	1	50
26	95,0	1	1	50
27	94,1	7	5	750
28	100,0	21	10	2 115
29	100,2	6	6	710
30	101,5	6	5	815
32	108,5	2	2	100
33	93,0	1	1	50
34	101,5	2	2	200
35	105,0	2	2	200
36	106,6	3	3	350
41	113,0	1	1	50

given in the table represent relative values, which are either lower or higher than 100, the average of the 28-class. When combining different data, belonging to the same chromosome class, consideration was paid to the number of pollen grains measured in each case.

As is evident from the values in Table 24 and the pollen curve represented in Diagram 3, *there is a very clear average increase of the pollen size as the chromosome numbers change from 14 to 41.* A few values deviate markedly from the regression line, but these values are based on the measurement of relatively few pollen grains. With regard

to the interval 15 to 21 the irregular shape of the curve is certainly also caused by the fact that the pollen grains measured represent the low percentage of apparently good grains found in non-dehiscing an-

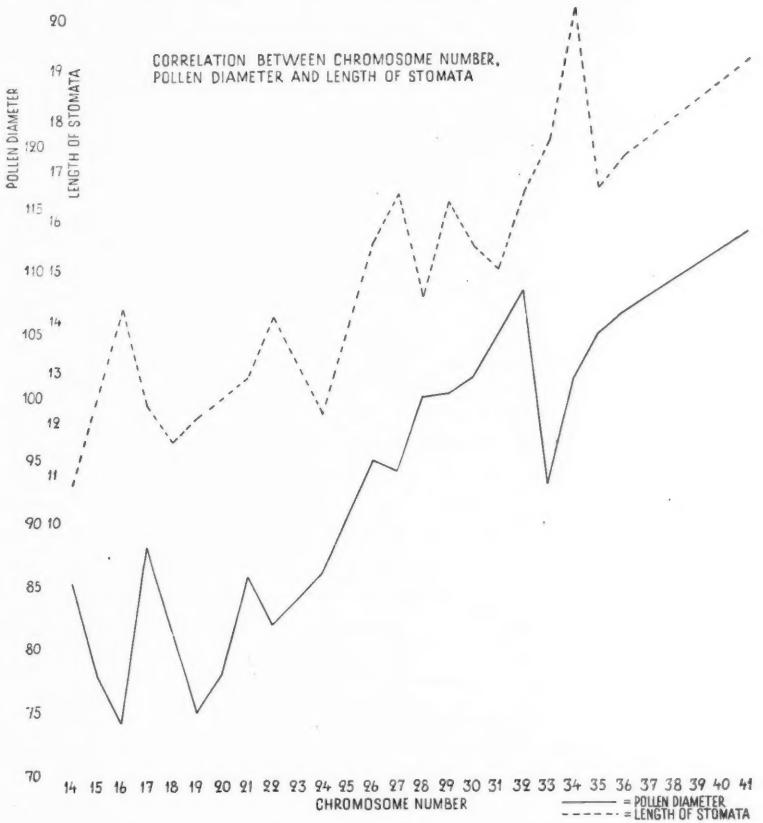

Diagram 3.

thers. As mentioned above all plants with 15 to 21 chromosomes were male sterile.

B. LENGTH OF STOMATA.

The measurements of stomata were all performed in the same summer and are thus comparable. Some of the plants, however, were greenhouse plants, the others were growing in the field. In spite of

this the two series of data obtained were found on the whole to be comparable and could be combined into the series represented in Table 25 and Diagram 3. As in the case of the pollen diameter, *there is quite a significant correlation between chromosome number and length of the stomata.* The regression is even greater in the present case, the average

TABLE 25. *Correlation between chromosome number and size of stomata.*

Chromosome number	Average length of stomata	Number of tests	Number of plants examined	Total number of stomata measured
14	10,70	6	3	300
16	14,24	1	1	50
17	12,34	1	1	50
18	11,62	1	1	50
19	12,08	1	1	50
21	12,88	6	6	300
22	14,06	1	1	50
24	12,18	1	1	50
25	13,84	1	1	50
26	15,56	1	1	50
27	16,54	3	3	150
28	14,47	7	4	350
29	16,38	3	3	150
30	15,50	3	3	150
31	15,04	1	1	50
32	16,53	2	1	100
33	17,56	1	1	50
34	20,20	1	1	50
35	16,66	1	1	50
36	17,28	1	1	50
41	19,22	1	1	50

values of the highest chromosome numbers being almost 100 per cent higher than those of the lowest classes. As is evident from Table 24, the corresponding increase of the pollen diameter is less than 50 per cent. The size of the stomata may consequently be considered to be a better index of the chromosome number than the size of the pollen grains. The occurrence of correlations between chromosome number and cell size has been reported from many other genera. In the present connection it seems superfluous, however, to go into a detailed discussion of this well-known phenomenon.

VI. DISCUSSION.

1. THE NATURE OF THE SPECIES DIFFERENCE GLOMERATA—ASCHERSONIANA.

From the cytological results, reported in the first part of this paper, it is evident that the genomes of *D. Aschersoniana* and *glomerata* are quite homologous. The high frequency of trivalents in the triploid *glomerata* × *Aschersoniana* hybrids shows that the *Aschersoniana* chromosomes are capable of complete conjugation with the *glomerata* genomes, and the high frequency of quadrivalents in *glomerata* demonstrates that the four genomes of this species are all of the same kind, at least from the cytological point of view. In short, *D. Aschersoniana* has two and *glomerata* four genomes of the same kind. The homology of these genomes could be further studied and verified in pentaploids obtained from the back cross triploid × *glomerata*. It is true that a continuous decrease in the frequency of multivalents with maximum association was found as the chromosome number increased from the diploid to the pentaploid condition. This decrease, however, is not caused by a reduced homology but by purely mechanical conditions, viz. the difficulty of a chromosome to maintain a connection with more than one other chromosome at a time, especially if the average chiasma number is low (NEWTON and DARLINGTON, 1929).

This cytological homology between the genomes of the two species is reflected by morphological differences, which are largely of a quantitative nature, the larger dimensions being represented by the species with the higher chromosome number. The quantitative nature of the morphological species differences could be directly demonstrated by an analysis of the experimental material, in which the chromosome numbers ranged from 14 to 41. Of the seven species differences studied only one was quite independent of the chromosome number, all the others showing a more or less pronounced correlation.

As was argued in detail above, the positive correlation observed between high chromosome numbers and *glomerata* morphology must mainly be a direct consequence of the higher chromosome number and cannot reasonably be attributed to specific genes present in one species and absent in the other. This conclusion was greatly supported by the fact that among the aberrants of *D. glomerata* those with the lowest chromosome numbers had a tendency to resemble *Aschersoniana*, those with the highest chromosome numbers, on the contrary, being super-*glomerata* types.

The connecting link between the chromosome numbers and the quantitative characters is certainly the cell size, which was found to be correlated with the chromosome number both with respect to the size of the pollen grains and the size of the stomata (Tables 24—25). There is not only a characteristic difference in cell size between *Aschersoniana* and *glomerata* but also the intermediate and higher chromosome numbers are correlated with the cell dimensions.

Under such circumstances it is quite comprehensible that *glomerata*, in contrast to *Aschersoniana*, is characterized by thick and tall culms, stiff and thick panicle branches, dark green leaves etc. Also the seeds of *glomerata* are evidently larger and heavier than those of *Aschersoniana*.

Dr. N. SYLVÉN has kindly informed me that in 1935 the thousand grain weight of one *Aschersoniana* biotype studied was 0,60 gr. The corresponding values of *all glomerata* biotypes tested at the sam time were higher and ranged from 0,69 to 1,33, the average value of 57 biotypes being 0,99. Some other previous data have also demonstrated that *Aschersoniana* has smaller seeds than *glomerata*.

Thus, *D. glomerata* presents most of the characters found to be typical of experimental autotetraploids, compared with their diploid original forms. A survey of these characters has been given previously (MÜNTZING, 1936 a, pp. 282—283).

When studying the correlation between chromosome number and morphology it was observed, however, that the degree of correlation varied in different characters, and in regard to the type of leaf sheaths there was no correlation at all. At least this character, and perhaps some of the other characters showing a weak correlation, must be controlled by specific genes. The occurrence of such species differences is also evident from the fact that plants with 28 chromosomes, collected in hybrid populations, were found to be morphologically less typical and more similar to *Aschersoniana* than other *glomerata* types (Table 18). There is also a certain intraspecific variation in *glomerata* as well as in *Aschersoniana* with respect to the same morphological species differences. If the species differences were exclusively caused by the quantitative change in chromosome number all 4n plants would have the morphological value + 7 and all 2n plants would be — 7. This is not so, however, as is evident from Table 18. It should also be observed with regard to all the seven species differences that the plants with 28 chromosomes in the *glomerata* as well as in the triploid × *glomerata* material (Tables 21—22) were often found to be 0 or — plants, although in the majority of the cases they were + individuals.

Consequently, although at least one of the species characters is independent of the chromosome number, nevertheless such characters may predominantly be associated with one or the other species, owing to selective correlation (TEDIN, 1925) of some kind. Even a purely quantitative change in chromosome number generally leads to a marked change in the ecological properties of the new type (MÜNTZING, 1936 a). If a species difference of the kind discussed has an ecological value, it will probably not fit equally well into the different physiological systems of the diploids and the tetraploids but will probably be more successful in one of them. Such may be the case, for instance, with the smooth sheaths characteristic of *Aschersoniana*. Though this character showed no correlation with the chromosome numbers in the experimental material, it is nevertheless much more frequent in the diploid than in the tetraploid species.

The question may be raised as to which species characters are most essential, those which are caused by specific genes, usually present in one of the species owing to selective correlation, or those directly caused by the difference in chromosome number. From the point of view of constancy, the latter type of species differences is more reliable than the other type. If all species differences between *Aschersoniana* and *glomerata* were upheld only by selective correlation, it is very doubtful if the two types could be distinguished as separate units, having the taxonomical rank of a species or subspecies. That this has been possible in the present case must in the first place be attributed to the direct consequences of the changed chromosome number, i. e. especially vigour, type of panicle and leaf colour, which constitute the main habitual difference between the two species. It is true that the gene controlled differences, e. g. the smooth or scabrid sheaths, are of a more qualitative nature, but this fact in itself does not show that they are the expression of something deep and fundamental in the constitution of the species.

The essential species criterion in the present case, as in many others, is represented by the great sterility of the F_1-hybrids and the segregation of types in the following generations, which are more or less unviable and sterile. This, however, is not caused by an unfortunate recombination of genes, which are specific for each of the species, but by purely quantitative conditions. Such disturbances are well known in experimental autopolyploids. Autotriploids are more or less sterile from purely numerical causes, and the aneuploid progeny

shows a reduction in viability and fertility, which is proportionate to the degree of unbalance.

It is true that in the present case, in contrast to the experimental autopolyploids, one or more morphological differences are controlled by genes, which are usually present in one of the species and not in the other. There is no reason to assume, however, that these differences are especially important, because they occur parallel with a quantitative barrier of sterility and unviability. Probably these morphological characters are not more important than the morphological differences between different ecotypes. Let us assume, for instance, that the chromosome number is doubled in one of two ecotypes, differing in one or more morphological characters. After the doubling of the chromosome number the two types will be separated by a barrier of sterility and unviability. On account of this barrier the same morphological differences would certainly be raised to the rank of species characters, though they have really nothing to do with the discontinuity created by the quantitative change.

It is obvious that the two *Dactylis* species are ecologically different, though this has not yet been sufficiently studied. *D. Aschersoniana* is a typical forest plant which, at least in South Sweden, grows only in the shady beech woods, *glomerata* is usually found growing in meadows and on the sides of ditches but seldom in woods. Further, they have evidently a different geographical distribution, the area of *glomerata* extending much farther to the north than that of *Aschersoniana* (cf. SAMUELSSON, 1923; HOLMBERG, 1926; SYLVÉN, 1934; HYLANDER, 1936).

The floristical and experimental knowledge of *Dactylis glomerata* and *Aschersoniana* may be summarized in the statement that they are morphologically and ecologically different and, moreover, separated by a barrier of sterility and unviability. Under such circumstances both types certainly deserve the rank of a species or ecospecies, according to TURESSON's (1922, 1929 b) terminology. Further, since *glomerata* must be considered to be an autotetraploid, the genomes of which are homologous with those of *Aschersoniana*, the two species cannot be of the same age. In all probability the diploid species, *Aschersoniana*, is the older of the two, *glomerata* having arisen later on by chromosome doubling. It is not probable that the diploid *Dactylis* types, now growing in South Sweden, Denmark and Central Europe, are the actual progenitors of *D. glomerata*, but, nevertheless, the present *D. Aschersoniana* must be very closely related to the original forms of *glomerata*. The relations between the two *Dactylis* species are evidently

of just the same type as between the diploid and tetraploid races of *Biscutella laevigata* studied by MANTON (1934).

Thanks to the twin method of producing heteroploidy (cf. MÜNTZING, 1937) I recently got an excellent opportunity of verifying (or possibly modifying) the conclusions reached in the present paper as to the relationship between the two *Dactylis* species. A haploid plant was recently discovered among twin seedlings of *Dactylis glomerata*. This haploid *glomerata* has not yet flowered, but if it reaches this stage — probably next summer — a study of meiosis, fertility and morphology will be extremely interesting. It is to be expected that in most respects this haploid *glomerata* will behave like the diploid species, *Aschersoniana*.

2. THE GENERAL IMPORTANCE AND CONSEQUENCES OF QUANTITATIVE CHANGES IN CHROMOSOME NUMBER.

In a recent review (MÜNTZING, 1936 a) I called attention to the evolutionary importance of purely quantitative changes in chromosome number. The conclusions drawn in that paper were in the main based on the characteristic properties of a number of intra-specific chromosome races or pairs of closely related species, these types being members of polyploid series. The *Dactylis* species, discussed in detail in the present paper, evidently represent a rather typical example of this kind, inasmuch as they fulfil all the following characteristics found to be typical in such cases: a) Morphological differences mainly quantitative, positive correlation between chromosome number and vigour; b) Geographical distribution and ecology different; c) F_1-hybrids partially sterile; d) High frequency of quadrivalents in the tetraploid and of trivalents in the triploid hybrids.

To these main characteristics it should also be added that, as mentioned above, *D. glomerata* presents most of the morphological characters found to be typical of experimental autotetraploids, when compared with their diploid original forms. It has also been demonstrated that this quantitative increase is correlated with a greater cell size (Tables 24—25). Finally, like many other similar cases, *D. glomerata* has been found to be cytologically unstable. As shown above, the production of aberrants is a result of the autotetraploid condition and the presence of quadrivalents at meiosis.

The evidence is incomplete only in one respect, viz. as regards the results of experimental crosses between the two *Dactylis* species. As pointed out in the general discussion (MÜNTZING, 1936 a, pp. 300—304),

there is generally a more or less pronounced incompatibility between the members of a natural (or experimentally produced) autopolyploid series. On account of the technical difficulties in the present case no artificial crosses between *Aschersoniana* and *glomerata* have been attempted. It is very probable, however, that also in *Dactylis* there is a partial incompatibility between the diploid and tetraploid types. The fact that a marked zygotic selection is at work in the back cross triploid × *glomerata* (cf. above p. 144), leading to the death of the zygotes with lower chromosome numbers (and among them also the zygotes with 21 chromosomes), may be taken as indirect evidence that there is a barrier of incompatibility between *Aschersoniana* and *glomerata*. This barrier cannot be absolute, however, since spontaneous triploids may actually be formed in nature.

The conditions found in *Dactylis* necessarily raise the question as to what extent similar relationships between species and chromosome races occur in nature. This question has already been considered in detail (MÜNTZING, 1936 a), but since this review was written I have gathered some additional data, which may be discussed in the present paper. These data may consequently be regarded as a supplement to the cases already dealt with in the previous paper.

A. NEW MORPHOLOGICAL DATA.

Among recent cases of experimental autotetraploids the maize material studied by RANDOLPH (1935) should first be mentioned. The 4n plants, having 40 instead of 20 chromosomes, reach about the same height as normal maize plants, but the stalks are thicker and sturdier and the leaves broader and thicker. Also the tassels, the individual parts of the staminate inflorescence, the ears and the kernels are distinctly larger. The 1000-grain weight of the kernels was in fact found to be about 50 per cent greater in the tetraploids than in the diploids, the absolute values being 330,2 and 221,6 respectively. This quantitative increase is correlated with an increase in cell size, the stomata, epidermis cells and pollen grains being distinctly larger than in the diploid. Morphological changes of a similar type but less marked are also characteristic of triploid maize plants. According to EYSTER (1934, p. 328), the first triploid maize plant, found by RANDOLPH and MC CLINTOCK, was more vigorous and had a thicker culm, broader leaves, stouter tillers, larger anthers and distinctly larger microsporocytes than the diploid plants of the same culture. — Though no data on autotriploids were given in the previous paper (1936 a) it should be

mentioned that experimental autotriploids are generally gigas types of the same kind as the maize triploids.

To take another instance, the triploid forms of *Crepis capillaris* and *tectorum* (NAWASCHIN, 1929) were distinguished by notably increased dimensions of the cells and cell organs, including the fruits. In fact the entire plants were found to be somewhat larger than the diploids. In pineapples, just as in maize, the occurrence and properties of triploid and tetraploid varieties in addition to the diploid types have been described by COLLINS and KERNS (1935). According to these authors, the triploid plants and their various parts are larger than the diploids and the cells of the triploids have their volume increased by 40 per cent. In the tetraploid plants the increase in plant size of cells and parts of the plants was found to have been carried still further, and the volume of the tetraploid cells was 60 per cent greater than that of the diploid cells. In contrast to these cases, in which the tetraploids are more vigorous than the diploids, the tetraploid *Citrus* types described by FROST (1926) have a somewhat lower vigour than the corresponding diploids. Also in this case, however, the tetraploids are characterized by broad, thick leaves and stout shoots.

The autopolyploids mentioned above, which have all arisen under experimental conditions, may be compared with some natural autopolyploids not recorded in the 1936 paper. Among wild autotriploids the case in *Populus tremula*, described by NILSSON-EHLE (1936) and MÜNTZING (1936 b), may first be mentioned. This aspen type, evidently, is exactly comparable to experimental autotriploids and is a marked gigas type with rather high stems, large and thick leaves and enlarged floral parts (male catkins and anthers). Also the stomata and pollen grains were found to be larger than in diploid types of the same species. A rather analogous autotriploid in the genus *Thea* has been studied by KARASAWA (1932 a). This type, described under the name of *Thea sinensis* var. *macrophylla*, grows wild in mountainous parts of Japan but is also frequently cultivated. As already indicated by the name, this type has larger organs as compared with diploids, and was also observed to have larger stomata.

Another case of intraspecific polyploidy correlated with quantitative morphological changes has been described by the same author (KARASAWA, 1932 b). In three types of *Crocus vernus* with the chromosome numbers 16, 24 and 32 the average leaf breadth was found to be 0,75, 0,98 and 1,30 cm. respectively. Finally, in the *Tradescantia* species, studied by ANDERSON and SAX (1936), the doubling of the

chromosome number has an unusually slight effect but, nevertheless, also in this case the plants of the tetraploid races are, on the whole, decidedly larger than the corresponding diploids.

B. PHYSIOLOGICAL EFFECTS OF CHROMOSOME DOUBLING.

As pointed out in the 1936 paper (pp. 293—300), autopolyploidy is not only accompanied by quantitative morphological changes but also by physiological alterations, affecting, e. g. rate of cell division, osmotic pressure, vitamin content, assimilation intensity, winter hardiness etc. It was also emphasized that these conditions in experimental autopolyploids agree very closely with the ecological differentiation invariably found between intra-specific chromosome races. These conclusions are supported on the whole by some recent or not previously mentioned papers. Thus, the tetraploid *Tradescantias* have a longer period of flowering than the diploids. They are also characterized by a greater colonizing ability and a much wider and more northern distribution (ANDERSON and SAX, 1936). The tetraploid *Citrus* types studied by FROST (1926) were remarkable for their slowness to bloom, and a similar situation is also met with in *Prunus* and *Pyrus*. According to CRANE (1936), diploids and polyploids in these genera behave differently with regard to the early seedling growth. In Plums, Apples and Pears, which are polyploids (or secondary polyploids), there is a prolonged juvenile period of growth, which seedlings have to grow out before flowers and fruits are formed. In seedlings of the diploid sweet Cherry, on the other hand, the development is much more rapid.

As demonstrated in the previous paper (1936 a, pp. 340—356), the retarded development in polyploids must be connected with the fact that perennial species generally have higher chromosome numbers than their annual relatives. It has also been pointed out by several workers that a northern distribution is often combined with polyploidy (cf. HAGERUP, 1928; TISCHLER, 1935 and MÜNTZING, 1936 a, p. 292). These two facts are in excellent agreement with the conclusion of KIRCHNER (1908, cited from STÄHLIN, 1929) that the original forms of many perennial species with a northern distribution are to be found among annual species with a southern distribution. It is highly probable that such southern species are characterized by lower chromosome numbers than the other category.

Some new data, constituting more or less marked exceptions to the rules discussed above, should also be mentioned. According to NISHIYAMA (1934), the flowering time of the autotetraploid *Avena* type,

studied by this author, was not changed by doubling the chromosome number, and in *Dactylis* the diploid type, *Aschersoniana*, is even later in flowering than the tetraploid *glomerata*. This lateness, however, may be of a secondary nature. — Recently SCHLÖSSER (1936 a) has obtained experimental results, which so far are in a marked contrast to the observed correlation between polyploidy and a northern geographical distribution. Autotetraploid forms of *Brassica Rapa* and two wild *Solanum* species were found to have less good winter hardiness and a lower osmotic pressure than the corresponding diploids. These results emphasize the necessity of making similar experiments with other kinds of material.

C. MEIOSIS.

Under this heading it should be emphasized once more that absence or low frequency of multivalents at meiosis in a polyploid is no reliable criterion of incomplete homology between the genomes (cf. the discussion in the 1936 paper, pp. 304—313, and LILIENFELD, 1936, p. 122). Even in experimental autopolyploids the maximum chromosome association possible is very seldom realized. This failure may chiefly be due to a limited chiasma frequency but also to an incomplete association of the homologous chromosomes at pachytene. According to DARLINGTON (1931), chromosome pairing in polyploids depends on *two* variables: frequency of exchanges of partners amongst chromosomes at pachytene and amongst chromatids at diplotene.

Some extreme instances of a low or completely lacking formation of multivalents are reported concerning some autopolyploids in the genus *Nicotiana*. According to GOODSPEED and AVERY (1929), a tetraploid race of *Nicotiana suaveolens* has 32 bivalents at meiosis, the diploid race having 16 bivalents. Further, in a triploid individual of *N. alata* most or all of the chromosomes were present as bivalents and univalents, no distinct trivalents being observed (AVERY, 1929). The author remarks that chromosome multiples larger than two do not ordinarily occur at I—M in *N. alata*, nor do they occur in *N. Tabacum* and *rustica*. In the genus *Solanum*, belonging to the same family, similar conditions are met with, the average frequency of multivalents in different polyploid *Solanum* types being rather low (cf. MÜNTZING, 1936 a, pp. 307—308 and AFIFY, 1936). This strengthens the suspicion that the polyploid types of *Nasturtium officinale* studied by MANTON (1935) are really autopolyploid, though there are no multivalents at meiosis. In view of such cases it is quite probable that many other

polyploids, regarded as allopolyploids on account of the absence of multivalents, are really autopolyploid. This seems to be the case with the hexaploid species *Phleum pratense*. On account of the results of GREGOR and SANSOME (1930) this species has been regarded as a good allopolyploid, and it has been observed to have 21 bivalents at meiosis (MÜNTZING, 1935). However, recent results by NORDENSKIÖLD (1937) demonstrate that at least two of the genomes are homologous with each other and the third is homologous to the *nodosum* genome. Hybrids with 28 chromosomes between hexaploid *pratense* and diploid *nodosum* were found to have regular meiosis and quite good fertility, thanks to autosyndesis. As a rule the chromosomes in these hybrids formed 14 bivalents but in a few cells a ring-shaped quadrivalent was also observed.

According to these results the constitution of *Phleum pratense* seems to be quite analogous to that of *Fragaria elatior* (LILIENFELD, 1933, 1936). Just as in *Phleum* there are no multivalents at meiosis in this species, though it has been demonstrated by various crosses that the three genomes of *Fragaria elatior* are all homologous *inter se* and homologous to the genome of the diploid species *F. nipponica*.

D. STERILITY AND INCOMPATIBILITY.

As pointed out previously (MÜNTZING, 1936 a, pp. 313—322), there is a rather marked contrast between polyploid chromosome races and experimental autotetraploids as regards the degree of fertility. Types belonging to the former category are generally quite fertile, in contrast to the experimental autotetraploids, in which fertility has been found to be more or less reduced. To explain this difference it was assumed (l. c. p. 319) that the natural tetraploids have good fertility owing to natural selection and possibly also due to secondary processes of various kind. It was also pointed out that the degree of sterility in the experimental autotetraploids varied considerably in different cases, thus indicating that in a larger material of the same kind natural selection might really be successful and lead to complete fertility. At the same time it was pointed out that the reduced fertility in experimental autotetraploids is not primarily caused by meiotic irregularities but is in the first place controlled by the genotypical constitution of the material in question.

These arguments are strengthened by some recent results. Thus, the maize tetraploids studied by RANDOLPH (1935) have evidently better fertility than most or all other autotetraploids, experimentally produced.

In comparison with diploid stocks the amount of reduced fertility varied from 5—20 per cent. It is noteworthy that this relatively slight reduction in fertility is found in plants in which meiosis is rather irregular, leading to about 50 per cent of aberrants with deviating chromosome numbers in the progeny. On the other hand, the complete fertility characteristic of wild autotetraploids is evidently not quite universal. ANDERSON and SAX (1936) report that in *Tradescantia* the diploid species have generally more than 90 per cent good pollen, but in the tetraploid forms the pollen fertility is somewhat lower, ranging from 70 to about 90 per cent. — These two cases in fact demonstrate that there is really no sharp distinction between the degree of fertility in wild and experimental autotetraploids.

As regards the occurrence of a barrier of incompatibility between the different members of a polyploid series the striking similarity between experimental autopolyploids and wild chromosome races and species has already been emphasized (MÜNTZING, 1933 b, 1936 a). A new good example of such incompatibility is afforded by the tetraploid maize strains, which gave a very poor result in crosses with diploid maize (RANDOLPH, 1935). — In a recent paper SCHLÖSSER (1936 b) has made an important contribution to the understanding of this incompatibility as far as pollen tube growth is concerned. In 2n and 4n strains of *Solanum racemigerum* the osmotic pressure was found to be rather different and to be lower in the tetraploid. Crosses between 2n and 4n plants, grown under the same conditions, failed altogether and this was shown to be due to checked pollen tube growth. SCHLÖSSER, however, succeeded in modifying the osmotic pressure, the tetraploids being grown under dry conditions, the diploids in the opposite way, and after this modification the crosses gave positive result.

Incompatibility between tetraploids and diploids is certainly often due also to other disturbances than checked pollen tube growth. SCHLÖSSER's result, however, very nicely shows how a quantitative change in the chromosome number primarily causes a physiological change, the altered osmotic pressure, this change having the secondary effect of creating a barrier of incompatibility between the 2n and 4n strains.

E. SOME NEW CASES OF WILD AUTOPOLYPLOIDS.

Although in many cases of natural autopolyploidy there is a diploid type corresponding to the species or races with higher chromosome numbers, several other cases are known in which this diploid type is lacking or at least has not yet been detected. Some typical cases of

this kind, in which the autopolyploid constitution is evident from the mode of meiosis, have already been reported (MÜNTZING, 1936 a, pp. 334—336). A new beautiful example of the same type is represented by *Hordeum bulbosum* (VON BERG, 1936). Meiosis in this species is evidently of just the same type as in *Dactylis glomerata*. Though no corresponding diploid is known, the high frequency of quadrivalents is sufficient proof of the autopolyploid constitution.

In some other polyploid species there are only bivalents at meiosis, but hybridization experiments with other species nevertheless demonstrate that the genomes are largely homologous. *Fragaria elatior* and *Phleum pratense* have already been mentioned. Another case of just the same kind is represented by *Solanum nigrum*. In a hybrid between *nigrum* ($2n = 72$) and *S. nitidibaccatum* ($2n = 24$) the 48 chromosomes were observed to form 24 bivalents at meiosis (ELLISON, 1936), thus demonstrating (partial) autosyndesis of the *nigrum* chromosomes. Earlier, JØRGENSEN (1928) had shown that in haploid *nigrum* there are generally $12_{II} + 12_{I}$ at meiosis. Since true multivalents are relatively rare in *Solanum* this type of pairing does not prove that the species in question contains different, non-homologous genomes. ELLISON (l. c.) concludes that the chromosome complement of *S. nigrum* probably consists of an original chromosome set of 12, represented 6 times. It is also possible, however, that the original basic number is 6. In any case, *Solanum nigrum* must be mainly autopolyploid.

F. CONCLUSIONS.

Opinions as to the evolutionary importance of quantitative, autopolyploid chromosome changes will largely depend upon the way in which the term autopolyploidy is defined. With this conception some workers evidently understand the exclusive presence of more than two *genetically identical genomes*. If this definition is accepted, probably not a single one of the polyploid chromosome races and species discussed could be included in the autopolyploid category. However, as previously pointed out (MÜNTZING, 1936 a, p. 311), the term autopolyploid was originally taken in a rather wide sense and was used to indicate homology between different genomes and not identity. A definition was suggested (l. c.) by saying that the genomes of an autopolyploid are structurally identical though they may be genetically different. In connection with this structural identity the corresponding (homologous) chromosomes of different genomes have an equal capacity of pairing and crossing-over with each other in any combination. Allopolyploidy,

on the contrary, involves structural differences between some of the genomes. — In connection with the work on *Fragaria elatior*, LILIENFELD (1936) has also entered into the terminological question and has suggested the terms *iso-* and *anisoautopolyploidy* for cases of identical or genetically different, but otherwise homologous genomes.

Autopolyploidy of the latter kind is evidently characteristic of a rather large and important category of plants. *Dactylis glomerata* is a typical example and many other cases have been discussed in the present paper and especially in the previous review (MÜNTZING, 1936 a). It is probable that the majority of polyploid types occurring in nature are allopolyploid, but it should be observed that the number of races and species found to be autopolyploid is rapidly increasing. It is also quite probable that many allopolyploid species once arose as autopolyploids, which have later been differentiated by secondary structural changes. According to NISHIYAMA (1934), *Avena barbata* is probably an example of this kind. Allopolyploids may also arise from crosses between different autopolyploids (cf. MÜNTZING, 1936 a, p. 338). Finally, it was emphasized (l. c. pp. 361—365) and also by LILIENFELD (1936) that all allopolyploids must be regarded as partially autopolyploid. Consequently, the really important point is not the question whether the polyploid plant species are mainly auto- or allopolyploid but the fact that purely quantitative changes in chromosome number have a great evolutionary significance.

3. THE EFFECTS OF ANEUPLOID CHROMOSOME NUMBERS.

A. VIGOUR.

Just as the study of autopolyploid types goes back to *Oenothera Lamarckiana gigas* (GATES, 1909) the prototype of aneuploid plants is represented by *Oenothera lata* (cf. LEHMANN, 1922). Later, trisomic plants have been studied in many other genera, e. g. *Datura* (BLAKESLEE, 1921 a, 1921 b, 1930), *Matthiola* (FROST and MANN, 1924; FROST, 1927; MANN LESLEY and FROST, 1928), *Solanum* (LESLEY, 1928), *Crepis* (NAWASCHIN, 1929), *Zea* (MC CLINTOCK, 1929; RHOADES and MC CLINTOCK, 1935), *Antirrhinum* (STUBBE, 1934) and *Secale* (TAKAGI, 1935).

In all these cases the 2n + 1 condition has been observed to cause a decrease in vigour, but this reduction may be more or less pronounced, depending on the genes carried by the extra chromosome. In *Matthiola,* for instance, the trisomics »Large» and »Slender» were found to be more vigorous (and fertile) than the other trisomics studied, »Large» being almost as vigorous as normal plants (FROST, 1927).

Plants corresponding to the formula 2n + 2 or 2n + 1 + 1 have also been studied in *Datura* (BLAKESLEE, 1922), *Matthiola* (FROST, 1927; MANN LESLEY and FROST, 1928), *Zea* (MC CLINTOCK, 1929) and other genera and have all been found to be still more inferior in vigour than the simple trisomics. FROST is of opinion that in *Matthiola* only a minority of the tetrasomic plants survive beyond the embryonal stage. In *Datura* even a 2n + 1 + 1 + 1 plant has been obtained and reported to be of a very feeble growth (BLAKESLEE, 1930). In a cross between the triploid *Oenothera semigigas* and a diploid type, *Oenothera* (*biennis* × *Lamarckiana*) *velutina*, the chromosome numbers were found to vary between 14 and 20 (DE VRIES and BOEDYN, 1924). According to their vigour and fertility these plants could be divided into four groups, normal plants and three categories of increasing abnormality. Plants with 14—15 chromosomes were found to be more normal on the average than individuals with 2n = 16, and these plants in turn were better than those having 17—20 chromosomes. Thus, just as in the triploid × *Aschersoniana* cross, described in the present paper, the most intermediate chromosome numbers (17 and 18) were correlated with the highest degrees of abnormality. The same conditions were also met with in *Zea* (MC CLINTOCK, 1929). In 10 plants, obtained from the cross 3n × 2n, the chromosome numbers ranged from 21 to 27. The least vigorous plants were those having the highest number of extra chromosomes.

In true diploid plant species monosomic individuals are generally not viable and only known as chimaerical branches of otherwise diploid plants. *Nicotiana alata* (n = 9) is possibly exception to this rule, though it seems probable that some chromosomes or parts of chromosomes in this species may be reduplicated. GOODSPEED and AVERY (1929) have described 2n — 1 individuals of *N. alata*, which are not only viable but also show little if any alteration in external morphology. However, according to AVERY (1929), a certain monosomic *alata* type was less vigorous than normal diploids. The addition of seven chromosomes also resulted in significant alteration and the addition of four chromosomes produced a highly abnormal plant. — Monosomic types are also known in a few polyploid species and are in most cases reported to have a somewhat reduced viability. Such is the case, for instance, in the tetraploid species *Nicotiana Tabacum*. According to OLMO (1935), the monosomics of this species, studied by him, seem to be slightly less vigorous than their normal sibs and are retarded in time of development. In contrast to this, LAMMERTS (1932) reports that in *Nicotiana*

rustica, which is also tetraploid, monosomic individuals had as good a viability as the normal plants. If *two* chromosomes are lacking, as in certain derivatives of the tetraploid *Primula Kewensis* (NEWTON and PELLEW, 1929), the result may be plants with a greatly reduced size, slow growth and exaggerated morphological characters.

Not much is known about the relative vigour of tetraploid plants with one or several extra chromosomes. A trisomic type of *Nicotiana Tabacum*, having 49 instead of 48 chromosomes (CLAUSEN and GOODSPEED, 1924), was characterized by an increased flower size, but otherwise there was no important difference between normal and trisomic individuals. Finally, a tetraploid tomato plant with four extra chromosomes studied by SCHLÖSSER (1934) was found to be considerably weaker than the normal tetraploid sister plants.

Though not complete the examples given above suffice to give a general idea of the influence of extra or lacking chromosomes on viability. It has long been realized that this is a question of balance, the influence being much greater if the total chromosome number is low. Further, the more the chromosome complement departs from the multiples of the basic number the more severe are the disturbances. Finally, different species evidently react in a different way, some being more sensitive to alterations in chromosome number than others. Hyacinths, for instance, represent one extreme and appear to be equally successful with every chromosome number between 16 and 30 (NEWTON and DARLINGTON, 1929). *A priori* it is probable that autopolyploids are less sensitive to aneuploid numbers than allopolyploids. If there is little effect of extra or lacking chromosomes in an allopolyploid species (as e. g. *Nicotiana rustica*) this may be regarded as evidence that the genomes are not greatly differentiated.

For the present problem our *Dactylis* material is suitable in two respects, viz. the great number of chromosomal types available at the same time and the possibility of making exact measurements. As far as I know, vigour in all the other examples cited has been estimated by mere observation and, in any case, not by weighing the plants. Further, in *Dactylis* a considerable part of the work has been devoted to the interval between tetraploidy and pentaploidy, which has previously been studied very little. The main results obtained from the *Dactylis* material have already been summarized above (p. 176), but this summary may be amplified by the following conclusions: The euploid numbers 14, 21, 28 and 35 represent the best vigour, but the difference between the euploid and aneuploid numbers, which is extreme in the

interval 14—21, becomes less and less distinct as the chromosome number increases. This is chiefly due to purely numerical causes, the difference between 14 and 15, for instance, being much greater than that between 35 and 36. These numerical ratios, however, do not cover the whole truth. At least with regard to the ± tetraploid numbers it is evident that on the average the subtraction of one or a few chromosomes has a much more marked effect than the addition of the same number. A similar experience is reported by YARNELL (1931) with regard to 3n — 1, 3n and 3n + 1 types in *Fragaria*. The loss of a chromosome from the 3n balance seemed to cause more of an upset than the addition of an extra one.

In *Dactylis*, too, it is evident that specific chromosomes have different effects on vigour. Especially in one particular progeny, described above (p. 165), the tetraploid plants with extra chromosomes had excellent viability. If the mode of meiosis in these plants could be stabilized new basic numbers might easily arise. Such a transition would indeed be rather easy in plants with small chromosomes and a low chiasma frequency. This problem has already been discussed more in detail in an earlier paper (MÜNTZING, 1936 a, pp. 356—361). The present results in *Dactylis* afford further support to the conclusion that autopolyploidy may facilitate the origin of new basic numbers. As the disturbing effect of aneuploidy was found to decrease rather rapidly as the total chromosome number increases, it is also quite comprehensible that species with high chromosome numbers may not be stable but have oscillating and aneuploid chromosome numbers.

B. FERTILITY.

As is well known aneuploidy is also correlated with a reduced fertility. These conditions have been studied exceedingly well in the 2n + 1 forms of *Datura* and other genera. In general the extra chromosome in such plants is not transmitted through the pollen, or at least only rarely. Transmittance on the female side is much better, but part of the ovules and zygotes containing the extra chromosome are eliminated and this causes a more or less marked decrease in seed production. The non-functional pollen is either visibly aborted or apparently normal but with abnormal germination or abnormal pollen tube growth. Finally, different chromosomes (or even parts of chromosomes) have somewhat different effects, precisely as in the case of vigour.

In *Datura* it is obvious that at least one chromosome of each kind

must be present in order to give viable gametes (BLAKESLEE, 1930). Duplications disturb the functioning of the gametes to different degrees, but deficiencies are directly lethal and result in aborted pollen grains and ovules. Deficiencies, however, may be transmitted by the sex cells of 4n plants. Duplications are also transmitted to a greater extent if the chromosome number is high. In *Datura* 4n + 1 Globe transmits the Globe character to a higher percentage of the progeny than 2n + 1 Globe (BLAKESLEE, 1922). Consequently although aneuploidy may have a less disturbing effect if the chromosome number is high, other cases of chromosomal aberrants in polyploid species are known, in which there is still a marked reduction in fertility. In the monosomic types of *Nicotiana Tabacum*, OLMO (1935) finds that the seed production is more or less reduced. Also the viability of the pollen is lowered though the percentage of shrivelled grains is low. LAMMERTS (1932) has made similar experiences with regard to monosomic types of *Nicotiana rustica*. Pollen grains with 23 instead of 24 chromosomes were as a rule non-functional and only three monosomic types were transmitted in appreciable frequency through the pollen. — Before discussing the findings in *Dactylis* it should finally be mentioned that a 4n + 4 type of *Solanum Lycopersicum*, studied by SCHLÖSSER (1934), was at first found to be markedly sterile, but the fertility could be very much improved by means of some generations of vegetative reproduction. The same improvement could be obtained also in plants with exactly 48 chromosomes (4n). These results furnish clear evidence that sterility in aneuploids (and autopolyploids) may also be diplontic and not only haplontic. In the same way the abnormal and almost empty anthers of *Oenothera lata* are an expression of diplontic sterility, since other 2n + 1 types in *Oenothera* have normal anthers and rather good pollen (cf. LEHMANN, 1922).

In *Dactylis* all the aneuploid plants with 15—20 chromosomes were found to be completely male sterile with non-dehiscing anthers and that was the case also with the triploid hybrids. In striking contrast to their triploid mother plants the pentaploid derivatives were found to have very good fertility, the average percentage of good pollen in this class being 93,2. Seed production in the pentaploid hybrids is very good too. Owing to this difference and the continuous increase in pollen fertility as the chromosome numbers increased from 22 to the higher numbers, the poor fertility in the triploids may be explained in the following way. Most of the gametes receive aneuploid

chromosome numbers, and since these numbers are generally low, the unbalance is very marked and causes the death of the majority of the pollen grains. This haplontic sterility has a secondary effect, the non-dehiscing anthers. Just as in wheat hybrids and other grasses the anthers must certainly contain a minimum of normal pollen grains in order to dehisce. This percentage is too low in the triploids but more than sufficient in the pentaploids. In spite of very irregular meiosis, leading to pollen grains with widely different chromosome numbers, most of these grains are viable owing to their autopolyploid constitution, i. e. the reduplication of homologous chromosomes.

Judging from the curve of pollen fertility (Diagram 2, p. 188) the maximal pollen fertility is reached already a few classes above 28, and after this the curve evidently remains mainly horizontal. A similar correlation between fertility and chromosome number has been observed by LEVAN (1936) in *Allium nutans* and *A. Schoenoprasum*. In this material there was a positive correlation between chromosome number and seed production after isolation. The author concludes that this correlation is brought about by an increasing self-fertility. This is probably true in part, but, in view of the *Dactylis* results, it is obvious that much of this correlation must be due simply to a better viability of gametes and zygotes with high chromosome numbers.

In *Dactylis* fertility on the female side has not been studied to any great extent, but from the observations made it is clear that the ovules are much less sensitive to aneuploid chromosome numbers than the pollen grains. This is quite natural since the pollen grains during their short but independent life are subject to more variable and severe environmental conditions than the sheltered female gametophytes.

4. THE POSSIBILITY OF PRODUCING HEXAPLOID DACTYLIS STRAINS.

The present *Dactylis* work was started for the purpose of producing new types with higher chromosome numbers than the tetraploid species *glomerata*. As the triploid *glomerata* × *Aschersoniana* hybrids were male sterile they could not give rise to polyploid progeny by self-fertilisation. The occurrence of unreduced, functional ovules, however, caused the production of a rather high proportion of pentaploids in the back cross with *glomerata*. These 5n plants were highly fertile and could be selfed or crossed with other pentaploids. In the progenies thus obtained there was a marked average decrease in

chromosome number, but some plants had higher numbers than the mother individuals. Plants with 35—41 chromosomes were also obtained directly from the cross triploid × *glomerata*. As these plants have quite good fertility they may be used as mother individuals of new progenies. In these progenies some plants will certainly have 42 chromosomes and, theoretically, some of them should be real euploid 6n plants.

The production of such plants and the preservation of hexaploid strains will largely depend on the strength of those selective influences which distinguish euploids and aneuploids. That is, the exactly hexaploid constitution must be correlated with a higher degree of viability than the surrounding aneuploid numbers. As demonstrated in the present paper this situation is typical of the tetraploid number. Though not quite stable the tetraploid species, *glomerata*, is preserved on account of the inferior viability and fertility of the aberrants with lower or higher numbers. In the progenies of such aberrants there was a marked tendency to revert to 28, the typical chromosome number of the species.

It is doubtful if the 6n condition can be upheld in the same way. As demonstrated above the differences in viability and fertility between euploids and aneuploids become less and less distinct as the chromosome number increases. As regards pollen fertility, a slight but significant minimum was found to be situated between 28 and 35, but above this minimum most plants seemed to have reached complete pollen fertility. The data hitherto gathered are insufficient to show whether there are further minima between 35 and 42 and above 42. Judging from the negative correlation between chromosome number and effect of aneuploidy, these minima should at any rate be very slight. However, as apparently normal pollen grains may be quite incapable of functioning there is, nevertheless, a possibility that 3n pollen grains may be more functional than 3n — 1 and 3n + 1 grains. Fertility on the female side will probably be still less influenced by deviations from the hexaploid number.

With regard to vigour the best pentaploids were very well developed and had a higher weight than most tetraploids. As shown above, there was a rather clear decrease in vigour between 28 and 35. Hitherto only a few plants with higher numbers than 36 have been obtained. Since the vigour of these plants is rather poor it seems probable, however, that there is another minimum between 35 and 42. It is also possible that these plants exceed the optimal chromosome

number, and that the vigour of all individuals with more than the pentaploid number will be more or less poor. The unviability of the plant with 56 chromosomes is an indication in this direction.

In *D. glomerata* the mode of meiosis is rather regular in spite of the frequent occurrence of quadrivalents. If hexaploid plants can be obtained, meiosis in these individuals will probably be more irregular. Theoretically, hexivalent groups should be frequent but, considering the meiotic behaviour in the pentaploid and in the plant with $2n = 41$, it is probable that most chromosomes will occur in smaller associations. This will probably cause a good many meiotic irregularities. On account of the autopolyploid constitution gametes with deviating chromosome numbers will probably be functional and the resulting aberrant plants will have a rather good viability and fertility. Thus, the immediate effects of meiotic irregularities in the hexaploids may be expected to be rather slight. In the long run, however, such irregularities may have dangerous consequences. If the difference in viability between the euploid and aneuploid numbers is too slight, the hexaploid strains cannot be upheld but will revert to the tetraploid number. Future research may elucidate this question and decide whether it is possible to extend the multiple series in *Dactylis* from tetraploidy to hexaploidy.

SUMMARY.

1) Meiosis has been studied in three biotypes of the diploid species *Dactylis Aschersoniana*. One of these types had regular meiosis, the second one contained a large fragment and the third was found to be heterozygous for a segmental interchange, causing an association of four chromosomes in some cells.

2) A study of the chiasma frequency in the segmental interchange heterozygote revealed the presence of a positive correlation between the total chiasma frequency and the occurrence of quadrivalent associations. In spite of the structural differences this biotype had the same average chiasma frequency as the other biotypes. This is probably an expression of a tendency to unitary control of the chiasma number.

3) The tetraploid species, *D. glomerata*, is not cytologically stable, at least 10 per cent of the individuals having aberrant chromosome numbers. This is a consequence of the high frequency of quadrivalents present at meiosis.

4) Meiosis in some aberrants of *glomerata* was found to proceed along the same main lines as in pure *glomerata*, but the odd chromosome numbers of aberrants with 27 and 29 chromosomes caused the formation of univalents, trivalents and quinquevalents in addition to the bivalents and quadrivalents. Significant differences in meiotic behaviour were found between different pentasomic individuals.

5) In progenies of *glomerata* aberrants there was a general tendency to revert to the normal chromosome number of *glomerata*. This tendency, however, varied in degree in different progenies and was evidently dependent on the quality of the particular chromosome involved and on the mode of meiosis.

6) Meiosis in triploid *glomerata* × *Aschersoniana* hybrids was studied in detail and was found to be mainly characterized by a high frequency of trivalents.

7) The triploid hybrids were male sterile but progeny could be obtained in back crosses with both parent species. The cross triploid × *Aschersoniana* resulted in plants with all chromosome numbers between 2n and 3n. The other combination, triploid × *glomerata*, gave a considerable proportion of ± pentaploid plants in addition to a majority of individuals with lower chromosome numbers. The pentaploids arise from the union of reduced male and unreduced female gametes. These unreduced ovules may sometimes contain one or several chromosomes more or less than the somatic number. Owing to selective influences the chromosome numbers of the plants from reduced gametes had higher chromosome numbers than expected.

8) Meiosis in a pentaploid plant was found to be irregular and was characterized by the presence of a variable number of quinquevalents, quadrivalents, trivalents, bivalents and univalents.

9) In progenies of pentaploids the chromosome numbers were quite variable, ranging from 28 to 41. On account of chromosome elimination the average chromosome numbers of these progenies were all lower than the chromosome numbers of the mother plants.

10) A study of the chiasma frequencies in the polyploid *Dactylis* types showed that the frequency per chromosome was the same in the tetraploid as in the diploid types. The pentaploid and especially the triploid were found to have a much lower chiasma number per chromosome. This is not due to incomplete homology but is associated with the fact that trivalents and quinquevalents are not capable of

forming closed rings. In the triploid as well as in the tetraploid there is a negative correlation between the chiasma frequencies of those chromosomes present as bivalents and those present as multivalents.

11) A study of the correlation between chromosome number and plant weight in various progenies revealed the following main results. The euploid numbers 14, 21 and 28 represent maxima of increasing size, the plants with 28 chromosomes being at least twice as vigorous as the plants with 14 chromosomes, and the triploids with 21 chromosomes being intermediate. With a high degree of probability there is a fourth maximum at 35, the pentaploid number.

12) The aneuploid numbers 15—20 represent a very deep minimum, the plants with 16—18 chromosomes scarcely being viable. A second minimum, situated between 21 and 28, is rather marked but less extreme than the first one. A third, less clear minimum is situated between 28 and 35.

13) The plants with 14 and 28 chromosomes were found to have complete or almost complete pollen fertility. The intermediate chromosome numbers are represented by male sterile plants (the interval 15 to 21) and individuals showing different degrees of partial sterility (the interval 22 to 27). There is a marked and significant increase of pollen fertility as the chromosome numbers increase from 22 to 28. There is a second significant but rather slight minimum between 28 and 35. Shortly above 28 the pollen fertility seems to have reached its maximum and in spite of irregular meiosis most plants with high chromosome numbers, including the pentaploids, have perfectly good pollen.

14) In progenies of *D. glomerata* and triploid $\times$ *glomerata* seed production was significantly higher in the plants with 28 chromosomes than in the aneuploid or pentaploid individuals.

15) In the spontaneous as well as in the experimental material a detailed study of the morphological species characters was undertaken. From this study it is evident that the morphological species differences between *Aschersoniana* and *glomerata* are mainly caused by the quantitative difference in chromosome number and not by specific genes present in one of the species and absent in the other.

16) Many of the morphological characters are probably greatly influenced by changes in cell size. Measurements of pollen grains and stomata revealed the occurrence of a marked positive correlation between chromosome number and cell dimensions.

17) Some further data are given concerning the evolutionary importance of quantitative chromosome changes. Finally, the effects of aneuploid chromosome numbers and the possibility of raising hexaploid *Dactylis* strains are discussed.

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EINE PRÜFUNG DER WEGE UND THEORIEN DER INZUCHT

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DASS man bei den obligaten Fremdbefruchtern mit einer Schwächung der Nachkommenschaft nach erzwungener Selbstbefruchtung zu tun hat, ist ja eine alte Erfahrung, die durch die umfassenden Versuche von DARWIN endgültig festgelegt wurde. Diese Verminderung der Vitalität, diese Depression, wurde auch als eine für die allogamen Pflanzen allgemeine, in ihrer Konstitution begründete Erscheinung angesehen.

Die mendelsche Forschung konnte die Depression nicht nur bestätigen, sondern noch stärker festlegen. Aber natürlich ist auch, dass man die Depression als den Effekt mendelnder Faktoren auffassen wollte. Namentlich die Versuche und Auseinandersetzungen von SHULL (1909, 1910, 1911), EAST and HAYES (1911, 1912) und EAST and JONES (1919) bezweckten, eine restlose Erklärung der Inzucht auf mendelscher Basis zu geben. Ihre Ansichten werden jetzt wohl allgemein anerkannt, ob für die praktische Züchtung vorteilhaft, werden meine unten vorgelegten Resultate von mehr als zwanzigjährigen Versuchen mit der Inzucht von Roggen zeigen. Da es mir augenblicklich wegen anderer Arbeitsaufgaben nicht möglich ist, meine Roggenversuche, die wohl nebst denen mit Mais die umfassendsten in bezug auf die Inzucht sind, ausführlich zu veröffentlichen, habe ich hier eine sehr gedrängte Zusammenfassung der theoretisch wichtigsten Resultate mitgeteilt. Denn die Antwort, die der Mais auf die Inzuchtfrage gegeben hat, gibt der Roggen nicht. Daher ergeben sich auch neue theoretische Probleme.

Als ich im Jahre 1911 mit meinen Roggenversuchen begann, war ich davon überzeugt, dass die Resultate von SHULL und EAST in bezug auf den Mais auch für andere Fremdbestäuber, also auch für den Roggen, gültig seien. Ihre Auffassung, dass die Ursache der Depression eine bei der Inzucht zunehmende Homozygotie ist, die durch Kreuzung wieder restituiert werden kann, schien die Grundlage für eine erfolgreiche Züchtungstheorie bilden zu können. Denn durch Isolierung und Selbstbefruchtung kann eine wertvolle Eigenschaft zur Konstanz gebracht werden, wenn auch mit Abnehmen der Vitalität, durch Kreu-

zung mit einer anderen isolierten, also gleichfalls deprimierten Linie, kann die Vitalität zurückerhalten werden, und man hat hierdurch die gewünschte Eigenschaft aus einer Population vital extrahiert. Man braucht nicht zu fürchten, diesen Weg einzuschlagen. Die Heterozygotie, die Heterosis, ist wieder hinreichend hergestellt.

Eine derartige Zickzackzüchtung habe ich nun beim Roggen in grossem Massstabe versucht. Dass die von mir zur Isolierung ausgelesenen Mutterpflanzen (Elitepflanzen) durchschnittlich die dem Aussehen nach besten waren, ist ja selbstverständlich, da ich eine Ertragssteigerung beabsichtigte. Ich habe also eine Plusauslese vorgenommen. Es fragt sich also nun, ob die F_2 oder die folgenden Generationen nach Kreuzung dieser isolierten Linien *durchschnittlich* einen so guten Ertrag wie die Ausgangspopulation geben werden, während sich einige als Plusvarianten, andere als Minusvarianten verteilen. Oder verteilen sich die Nachkommenschaften, die aus Kreuzung zweier Isolierungslinien stammen, in anderer Weise? In der folgenden Darstellung bezeichne ich eine Nachkommenschaft, die aus einer Kreuzung von Pflanzen, die früher eine Isolierung, also eine erzwungene Selbstbefruchtung durchgemacht haben, mit IK, Isolierungskreuzungen.

Die Antwort auf die aufgeworfene Frage gibt Tabelle 1 in der Kolumne IK. Diese Antwort ist sehr drastisch. Denn *die Isolierungskreuzungen geben einen durchschnittlichen Ertrag, der nur 76,2 % von dem der Ausgangssorten beträgt,* die Petkuser- oder Brattingsborgsroggen sind, hochertragreiche Sorten, die in Schweden denselben Ertrag gegeben haben. In diesem und sämtlichen weiter zu erwähnenden Versuchen ist Petkuserrogen als Standardsorte (Vergleichssorte) benutzt. Sein Ertrag ist also gleich 100 % gesetzt.

Aus diesen Zahlen geht ganz eindeutig hervor, dass die IK bei weitem nicht den Ertrag der Elternsorten (also der Elternpopulation) erreichen. *Es besteht noch eine durchschnittliche Depression von nicht weniger als 24 %.* Jedoch habe ich in bezug auf die F_1 ganz denselben Effekt von Stimulation wie SHULL, EAST u. a. konstatieren können. Es ist zwar nicht zu erwarten, dass die F_1-Stimulation bestehend sein soll, falls sie eine Heterosis bezeichnet, denn diese ist in F_1 maximal und sinkt mit dem Zurückgehen der Heterozygotie in F_2 und den folgenden Generationen. Aber es ist zu erwarten, *dass der Ertrag der IK um den Mittelwert der Ausgangssorte pendeln soll.* Das ist nicht der Fall. Statt dessen geben sämtliche IK, eine einzige ausgenommen, einen Ertrag, der mit 6—46 % hinter dem der Ausgangssorten zurückbleibt. Dieses Faktum steht fest.

Durch eine Isolierungszüchtung kann man also bei den allogamen Pflanzen ebenso wie bei den autogamen eine scharfe Differenzierung

TABELLE 1. *Resultate in bezug auf den Ertrag verschiedener Inzuchttypen.* (*Der Ertrag der Ausgangssorte = 100.*)

IK	PK	K (Kreuzungen verschiedener Sorten)	EL	EK	GK
53,9	60,7	81,8 Brattingsborg × Heinrich	78,6	85,3	79,3
58 5	68,3	84,3 » »	78,7	88,4	88,8
64,2	74,9	84,9 Heines Kloster × Petkuser	82,6	88,8	89,1
64,4	76,6	86,0 » » Heinrich	85,4	89,9	90,0
66,7	77,2	88,6 Brattingsborg × Petkuser	86,1	92,1	91,3
67,8	78,2	89,5 Buhlendorfer × Heinrich	86,4	93,7	93,8
68,2	78,3	72,4	86,8	94,0	93,8
68,4	79,0	73,2	87,4	94,0	94,2
69,1	80,9	73,8	88,5	94,0	95,2
70,0	81,6	77,6	88,6	95,0	96,7
71,3	81,7	77,7	88,8	95,4	97,7
71,6	81,8	80,5	88,8	95,4	97,8
72,0	81,8	80,7	89,1	95,5	97,9
72,2	82,6	81,2	89,5	95,9	98,0
72,4	82,6	81,7 F_2-Linien aus Brattingsborg × Pet-	89,8	96,8	98,3
73,3	82,8	82,7 kuser	90,0	97,0	98,5
78,2	83,7	83,0	90,0	97,0	99,3
78,5	84,1	83,2	90,4	98,4	99,5
79,0	84,6	84,9	90,5	100,0	99,5
79,4	84,6	85,0	91,6	100,4	100,4
80,0	85,3	87,2	92,6	100,5	101,1
80,0	86,2	88,1	92,8	100,6	101,4
80,5	86,6	90,3	92,8	100,8	101,5
80,6	89,2	93,0	93,0	100,8	101,7
80,9	90,1	96,4	93,1	100,9	101,9
82,3	92,3	56,4	93,5	101,0	104,8
82,9	92,5	73,7	94,0	101,9	105,6
84,5	93,6	85,0	94,9	102,0	106,0
84,6	93,8	88,5 F_2-Linien aus Brattingsborg × Hein-	94,9	102,0	106,9
87,1	94,0	89,8 rich	95,1	104,6	
93,1	94,1	91,2	96,3	111,5	
94,2	96,0	92,8			
103,6	96,5	99,6			
	96,7				
76,2	84,5	83,8	89,7	97,2	97,6

des genischen Materials durch Isolierung erreichen. Man kann also steifhalmige, kurzhalmige, grünkörnige, auch gewiss potentiell ertrag-

reiche Linien züchten. Sie sind aber alle deprimiert. Man wünscht diese Eigenschaften durch Kreuzung ähnlicher Linien vital restituieren zu können. Dies gelingt aber nicht, jedenfalls nicht beim Roggen. Meine umfassenden Versuche, diesen Züchtungsweg zu betreten, sind gänzlich gescheitert. Ich brauche wohl nicht noch einmal zu betonen, dass ich in dieser Hinsicht kein Verurteil gehabt habe, sondern dass die fortschreitenden Versuche mich durch das Resultat gezwungen haben, die Theorie der Heterosis, von der ich als eine sehr wahrscheinliche Erklärung ausging, als Grundlage der Züchtungsarbeit aufzugeben. Denn bei theoretischer Forschung kann man wohl das Leben einer Lieblingstheorie hartnäckig zu verlängern suchen, das schadet oft nicht und zwingt zu Debatten und Verifizierung, bei praktischer Züchtung kann es nur als Unsinn betrachtet werden, wenn man nach einer Theorie arbeitet, wodurch nur Sorten mit einem geringeren Ertrag als dem der Elternsorte erhalten werden.

Es ist daher selbstverständlich, dass ich diese Züchtungsmethode, nämlich die IK-Züchtung aufgeben musste, obgleich dieser Weg der effektivste war, um das Material zu differenzieren. Sowohl den höchsten Grad genischer Differenzierung, nämlich die Isolierung einzelner Pflanzen, als den nächst höchsten, nämlich die Kreuzung zweier isolierten, ähnlichen Linien, muss man also beim Roggen aufgeben. Da auch im letzten Falle noch eine Depression bestehen bleibt, wäre es ja denkbar, dass die erzwungene Selbstbestäubung, d. h. die Vereinigung zweier Gameten derselben Pflanze, eine rein physiologische Störung verursache, die bestehen bleibt, auch wenn sie in F_1 durch den Heterosiseffekt verschleiert würde. Man wird jedenfalls vom Resultat der IK-Züchtung belehrt, jede Differenzierungsmethode zu vermeiden, die auf erzwungener Selbstbefruchtung, also reiner Autogamie, sich gründet.

Das nächste Stadium der Inzucht, wodurch man eine möglichst effektive Differenzierung, d. h. Einengung der Variabilität der Population (Ausgangssorte) in bestimmter Richtung, erzielen kann, ist eine Kreuzung zweier Pflanzen mit gewünschten Eigenschaften. Ich habe deshalb auch eine grosse Serie solcher Kreuzungen, also Paarkreuzungen, ausgeführt. Ich bezeichne diese mit PK. In Tabelle 1 habe ich einen Teil dieser Kreuzungen, nämlich die von den oben erwähnten Sorten abstammenden, zusammengestellt. Sie sind so ausgeführt, dass zwei Linien von Petkuserroggen, oder eine Linie dieser Sorte, eine andere von Brattingsborgsroggen, nebeneinander gepflanzt wurden. Von zwei Pflanzen jeder Linie wurden Ähren in derselben Pergamintüte vereinigt. Hierdurch wird eine Individualkreuzung dieser beiden Pflanzen

herbeigeführt (PK). Die Tüte hindert ja in diesem Falle nur ungewollte Bestäubung seitens anderer umgebender Pflanzen, aber es findet keine erzwungene Selbstbefruchtung statt. Eine normale Kreuzung findet von selbst statt, gerade wie man sie bei Autogamen künstlich nach Kastrierung ausführt.

Da die gekreuzten Pflanzen hier nicht näher verwandt sind, sondern aus einer sehr variablen Sorte, wie es alle Sorten des Roggens sind, oder sogar aus zwei sehr verschiedenen Sorten, wie es Petkuser- und Brattingsborgsroggen sind, stammen, ist kaum zu erwarten, dass dieser Differenzierungsgrad eine Depression verursachen wird. Jedenfalls war ich weit davon entfernt, einen solchen Effekt zu erwarten, wenn auch die Nachkommen einer derartigen Kreuzung Geschwister sind. Denn die alte Lehre von der Blutverwandtschaft als Ursache der Inzuchtwirkung hatte in meinem Kopf keinen Platz mehr.

Es war deshalb ganz überraschend, die Ertragsresultate der PK in F_2 oder den folgenden Generationen, wie sie in Tabelle 1 zu sehen sind, von Jahr zu Jahr feststellen zu können. Ich hatte ja die besten Linien der ertragreichsten Sorten gekreuzt, Sorten, deren Erträge ich schon kannte und die nie isoliert worden sind. Es wurden also Pflanzen benutzt, die eine kräftige Plusauslese bezeichneten. Und das Resultat? *Der durchschnittliche Ertrag betrug nur 84,5 % von dem der Elternsorten, und keine einzige PK-Nachkommenschaft erreichte den Ertrag der Eltern.* Also ist auch bei dieser Inzuchtmethode eine *kräftige Depression* zu konstatieren.

Dieses Konstatieren hat ja eine ganz paradoxale Konsequenz. Man kann nämlich beim Roggen nicht wie bei den autogamen Pflanzen mit Kreuzungen gewöhnlicher Art arbeiten. *Kreuzungen zweier Pflanzen, also Individualkreuzungen, lohnt es sich nicht als Ausgangspunkt einer Züchtungspopulation herzustellen.* Die PK-Nachkommenschaften der Tabelle sind ja derartige Kreuzungen. Zwar habe ich hier die Samen beider zusammen eingeschlossenen Ähren ausgesät, also die reziproken Kreuzungen zusammen, so dass die PK-Deszendenz (F_1) *zwei* Mütter hat, aber hierdurch wird ja die Inzucht nicht so gross wie bei einer Kreuzung, wo man die Ähre einer Pflanze mit dem Pollen einer anderen bestäubt und also nur *eine* Mutter zu F_1 hat. Wo man also als Ausgangspunkt eine einfache Individualkreuzung, ohne ihre Reziproke hat, nenne ich diese schlechtweg K. Meine Versuche haben nun gezeigt, dass man auch mit Kreuzungen dieser Art ganz vergebens arbeitet, was ja nach dem oben referierten Resultat mit PK natürlich erscheint.

Während der ersten Jahre meiner Züchtungsarbeit (1911 u. f.)

führte ich mehrere Kreuzungen zwischen den wichtigsten Handelssorten aus, wobei ich vor allem Petkuser- und Brattingsborgsroggen als die ertragreichsten Sorten benutzte, und ausserdem Heinrichroggen, um die Halmstärke zu verbessern. F_1 wurde als Population vermehrt und in F_2 (oder späteren F, die ja mit F_2 als Populationen gleichwertig sind) in bezug auf den Ertrag geprüft. Diese Populationen (K) zeigten alle einen sehr schlechten Ertrag, auch die Kreuzungen ertragreicher Sorten, wie Tabelle 1 in der dritten Kolumne, oben, zeigt. Ich glaubte daher, dass ich zufällig schlechte Eltern gewählt hatte, was man ja bei der grossen Variabilität der Elternsorten nicht sicher vermeiden kann. Dass ich aber *nur* minderwertige Eltern antraf, erschien indessen eigentümlich. Ich vermutete daher, dass die F_1-Synthese genisch ungeeignet ausgefallen war und wählte deshalb in F_2 die anscheinend besten Pflanzen aus und hoffte, unter ihnen jedenfalls einzelne anzutreffen, die Pluskombinationen darstellen. Dies traf aber nicht ein. Die Deszendenzen von F_2-Pflanzen waren durchschnittlich ebenso schlecht wie die F_1. Nur eine einzige F_2-Linie erreichte beinahe den Ertrag der Eltern, keine wies einen besseren Ertrag auf. Der Mittelwert sämtlicher F_1- und F_2-Linien aus K war nur 83,8 % vom Ertrag der Eltern. Diese Zahl stimmt überraschend gut mit der für die PK erhaltenen, nämlich 84,5 %, überein. So muss es auch sein. Nachdem ich 10 Jahre später die oben angegebenen PK-Resultate erhalten hatte, war auch das erbärmliche Resultat meiner Sortenkreuzungen klar. *Man kann also beim Roggen nicht mit Individualkreuzungen, weder mit K noch PK arbeiten, falls man praktische Resultate erzielen will.*

Zu Beginn meiner Züchtungsarbeit fing ich auch mit einer Auslese von einzelnen Pflanzen, Elitepflanzen an. Sie wurden als Elitedeszendenzen, Elitelinien, die ich deshalb als EL bezeichne, unter solchen Bedingungen (siehe unten S. 245) separat vermehrt, dass zwischen den Linien keine Kreuzung stattfinden konnte. Die Elitepflanze selbst ist einem Bestand, einer freien Population, entnommen worden, ist also einem reichen Durchkreuzen durch die Pollenwolke des Bestandes ausgesetzt gewesen. Ihre Nachkommen waren also Halbgeschwister und sehr heterozygot. Die Inzucht ist also hier weit milder als bei den PK. Kann nun auch bei diesem Inzuchtgrad eine Depression festgestellt werden?

Betrachtet man die vierte Kolumne von Tabelle 1, so erscheint die Antwort auch in diesem Falle ganz klar. *Der durchschnittliche Ertrag beträgt im Vergleich mit der Elternsorte nur 89,7 %, ist also mit 10 % erniedrigt. Die Depression ist also auch für die Inzuchtkategorie der*

EL ganz deutlich. Man kann daher in der praktischen Züchtung offenbar auch nicht mit Linien (Pedigrees) arbeiten, die von einer Elitepflanze abstammen, die mitten in einem Roggenfeld bei vollständig allogamer Befruchtung ausgewählt worden ist. Das war mir die dritte ebenso grosse wie unwillkommene Überraschung in bezug auf die Inzucht des Roggens.

Von diesen Positionen in bezug auf die Differenzierung des Materials, nämlich von den IK, PK und EL, allmählich verdrängt, musste ich, da ich den Hauptzweck jeder Züchtung, nämlich die genische Differenzierung, nicht aufgeben wollte, nach einem Weg suchen, auf dem ich diese möglichst effektiv machte, ohne Inzuchtschwächung zu erhalten. Denn zu einer Familienzüchtung überzugehen, bei der sämtliche Nachkommenschaften gleicher Abstammung zusammen abblühen, und Plus- und Minusfamilien sich druchkreuzen, so dass die Ausgangspopulation oder Muttersorte schlechtweg wieder restituiert wird, schien mir fast ebenso hoffungslos wie eine Isolierungszüchtung.

Es war ja schon klar, dass Individualkreuzungen ausgeschlossen sind. Aber ein anderer Kreuzungsweg würde vielleicht besser gelingen, nämlich eine Kreuzung von Elitelinien, den EL. Nachdem ich über die Depression der separaten EL ins Klare gekommen war, wählte ich auch diese Methode als die nächste, bei der die Inzucht noch milder gemacht wurde, aber die Differenzierung noch ziemlich stark war, weil ja die EL von einzelnen Pflanzen stammten und deshalb gewisse der Linien ziemlich ähnliche und ausgeglichene Bestände repräsentierten, weshalb sie auch ziemlich konform in einer Kreuzung vereinigt werden konnten. Diese Linienkreuzungen nenne ich EK, Elitekreuzungen.

Die EK sind also *keine Individualkreuzungen*, sondern *Bestandkreuzungen.* Sie werden so ausgeführt, dass die Körner zweier EL vor der Aussaat gemischt werden, sodass die Pflanzen der beiden Linien im aufwachsenden Bestand ganz gemischt stehen und sich deshalb auch effektiv durchkreuzen. Im ersten Jahr wird also die Bestandkreuzung spontan durchgeführt, aber nur partial, denn die Linienpflanzen benutzen teilweise ihren eigenen Pollen, und deshalb wird der Bestand nur zu 50 % durchgekreuzt. In der Deszendenz, die man wohl am besten mit D_1 bezeichnet, denn man kann hier kaum F_1 anwenden, wird das Durchkreuzen 75 %, in D_2 87,5 % und in D_3 93,75 % erreichen. Erst jetzt ist also die Durchkreuzung praktisch genommen vollständig, weshalb *die Prüfung in bezug auf den Ertrag erst in F_4 stattfinden darf.* Eine frühere Prüfung ist inexakt, weil noch ein deprimierter Teil der Elternlinien vorhanden ist. Ausserdem hat man, wie meine Versuche

mit D_1 und D_2 zeigen, bei den Elitekreuzungen auch mit einer ganz deutlichen Stimulation zu rechnen, die also auch ausklingen muss, ehe man eine stabile Ertragsfähigkeit erhält. Die EK-Bestände müssen natürlich sämtlich jedes Jahr separat gehalten werden, so dass sie nicht ungewollter Bestäubung von anderen Nachkommenschaften ausgesetzt werden.

Das Resultat der EK-Prüfungen, das in Kolumne 5 der Tabelle 1 wiedergegeben ist, ist von grösstem Interesse. Es *weicht nämlich ganz und gar von den Resultaten der IK, PK und EL ab.* Hier ist nämlich *keine Depression mehr zu sehen.* Hier verteilen sich nämlich die Varianten vollkommen nach dem Prinzip der Normalkurve, und hier liegt der durchschnittliche Ertrag der EL (97 %) sehr nahe demjenigen der Elternsorten (100 %). Die Depression der EL ist also durch ihre Bestandkreuzung ganz aufgehoben. *Nun spielt sich alles auf einem sozusagen genischen Plan ab.* Die Mehrzahl der EK geben einen Ertrag, der nahe dem der Eltern liegt. Man erhält Populationen mit geringerem Ertrag, aber auch solche, die ertragreicher als die Elternsorten sind. Alles verläuft so, wie bei einer Auslese von Pflanzen und bei einer Prüfung ihrer Nachkommenschaften bei Selbstbestäubern. Die Methode der Elitekreuzung ist deshalb eine praktisch verwertbare Methode.

Es ist also ganz offenbar, dass man beim Roggen nicht mit Isolierungskreuzungen, ja gar nicht mit Individualkreuzungen überhaupt züchterisch arbeiten kann. Der höchste Grad einer genischen Differenzierung, die ohne Depression durchgeführt werden kann, ist eine Auslese von Elitelinien und nachfolgende Elitekreuzung. Man kann natürlich auch von drei oder mehr Linien in der oben erwähnten Weise Bestandkreuzungen ausführen. Da man indessen hierbei nur die Differenzierung vermindert, und da man weiter schon bei der Kreuzung zweier Elitelinien die Depression aufhebt, besteht kein Grund, mit derartigen Beständen von gruppierten Elitelinien züchterisch zu arbeiten, denn diese Methode bietet keine Vorteile. Meine Versuche haben auch gezeigt, dass sie sich so nahe um den Ertrag der Elternsorte halten, aus der die zusammmengruppierten EL ausgelesen worden sind, dass diese schon bei 5—10 Linien rekonstruiert erscheint.

Eine weitere Inzuchtmethode bestünde darin, diese Gruppenkreuzung so auszuführen, dass man keine Samenmischung der EL vornimmt, sondern die EL nebeneinander in Reihen aussäht. Eine Bestandkreuzung findet auch dann statt. Die einzelnen EL wurden aber *später separat ausgelesen,* der Bastard also wieder aufgelöst. Auch in

diesem Fall wurde eine vielseitige Kreuzung der EL-Komponenten ausgeführt, aber diese dann wieder als EL ausgelesen und separat vermehrt. Da die EL unter sich eine Gruppenkreuzung durchgemacht haben, könnte man sie nun als Gruppenkreuzungen, GK, bezeichnen.

Auch diese Methode von »outbreeding» der Elitelinien durch gegenseitige Gruppenkreuzung, aber mit nachfolgendem Freimachen der Komponenten, scheint eine befriedigende Züchtungsmethode zu sein, wobei die Inzuchtdepression der EL aufgehoben worden ist. Das konnte man nicht voraussetzen. Denn die Transformierung der EL in GK bedeutet ja nur, dass die EL polyandrischer geworden sind, aber sie bilden fortwährend einen Halbgeschwisterkreis, denn in ihrer monogynen Struktur ist nichts geändert worden. Falls eine monogyne Abstammung einer Population immer mit Depression verbunden wäre, so sollte man sie auch bei GK finden, die nur reicher durchgekreuzt sind als EL, aber von ein und derselben Mutterpflanze abstammen.

Die Entscheidung bringen auch in diesem Falle die Versuchsresultate, die in der sechsten Kolumne von Tabelle 1 veranschaulicht werden. Sie zeigen ganz klar, dass *die gruppengekreuzten Elitelinien, die GK, keine Depression aufweisen. Sie folgen in bezug auf die Variation der Ertragszahlen sehr gut den EK*, variieren also um den Mittelwert der Elternsorte und geben deshalb unter den extremen Varianten auch solche, die diese Sorte übertreffen.

Das Resultat dieser Versuchsserien, in denen ich die Probleme möglichst scharf in Bezug zur Inzuchtfrage zu stellen versucht habe, dazu von dem geringen Erfolg der Isolierungszüchtung gezwungen, zeigen ganz offenbar, dass man es jedenfalls *beim Roggen mit einer Inzuchtwirkung zu tun hat, die nicht genischer Natur ist.* Denn erst wenn diese spezielle Depression aufgehoben worden ist, spielt sich alles so ab wie bei einer autogamen Pflanze. *Die Versuche zeigen dies mit einer Klarheit und Signifikanz, die wohl kaum besser gefordert werden kann.*

Ehe ich indessen zu einem zusammenfassenden experimentum crucis übergehe, das meine Auffassung noch strikter verifizieren wird, muss ich einige Worte über die Methodik der Durchführung meiner Versuche sagen, weil die Voraussetzung richtiger Resultate natürlich eine einwandfreie, reine Methodik ist. Zu Beginn meiner Versuche habe ich auch Experimente über die besonders wichtige Frage des Vizinismus angestellt (HERIBERT NILSSON, 1917).

Durch Benutzung eines pflanzlichen Indikators (wachsfreies Roggens) gelang es mir, gewöhnliche Isolierungsmittel zu prüfen und

die Pollenverbreitung nach Abstand, Windstärke, Windrichtung und Grösse der Bestände ganz exakt festzulegen. Hierbei konnte ich zeigen, dass Gewebehäuschen zwecks Isolierung von Pflanzen und Nachkommenschaften, die nebeneinander ausgesät werden, den Pollen zu 70 % durchlassen können, obgleich sie für das Auge ganz dicht aussehen. Die Maschenlöcher sind nämlich grösser als die Pollenkörner, und diese gehen daher wie Staub durch das »Isolierungsmittel». Die Nachkommenschaften von anemogamen Fremdbestäubern können in dieser Weise unmöglich separiert werden. Dann vermischt man nur alles und kann niemals zuverlässige Resultate erhalten.

Die erste Voraussetzung für eine Differenzierungszüchtung und für das Durchführen einer Untersuchung über die Inzucht ist natürlich, dass die Nachkommenschaften, seien sie IK, PK, EL, EK oder GK, *alle* je für sich *Jahr nach Jahr* gesondert gehalten werden, *separiert werden*, denn sonst weiss man ja nicht, womit man arbeitet, und die Ertragsresultate sagen über die Inzuchtwirkung gar nichts aus. Da man die Nachkommenschaften durch Gewebehäuschen nicht isolieren kann, habe ich sämtliche durch Abstand separiert. Ich habe sie nämlich an den Rändern von Weizenfeldern ausgesetzt. Bei der Grösse von 1 m^2 eines Bestandes habe ich einen Abstand von 30 m benutzt, bei 5 m^2 50 m, bei 10 m^2 75 m. Diese Abstände sind durch die erwähnten Indikatorversuche exakt ermittelt worden. Diese Separierung hat jedes Jahr eine beträchtliche Streckenlänge erfordert, 30—40000 m. Die Durchführung einer konsequenten Separierungszüchtung ist also eine mühvolle Aufgabe, aber notwendig, falls man wissen will, womit man arbeitet. Setzt man IK- und EL-Bestände nebeneinander in Gewebehäuschen, so arbeitet man nächstes Jahr *nicht mehr mit IK und EL*, sondern mit *partialen EK verschiedener Durchkreuzung*. Viele Resultate in bezug auf die Inzucht sind so entstanden!

Man kann auch nicht mit theoretischen Versuchen einer anemophilen Pflanzenart wie der Roggen in einer Gegend arbeiten, wo Roggen allgemein gebaut wird. Denn grosse Pollenwolken können dann alles aberrieren. Meine Versuche sind deshalb in die grossen Weizendistrikte Schonens verlegt worden (Landskrona, Hälsingborg, Alnarp).

Die Ermittelung des Ertrags stützt sich für sämtliche hier behandelten Zahlen auf feldmässige Versuche. Diese sind mit fünf Parallelparzellen von der Grösse 10 oder 20 m^2 ausgeführt. Standardsorte ist immer Petkuserrogen gewesen, weshalb sämtliche Versuche eines Jahres und auch verschiedener Jahre über diese Sorte verglichen werden könnten.

Meine Züchtungsmethode, die ich *Separierungszüchtung* nennen möchte, weil jede Nachkommenschaft durch Abstand separat gehalten wird, im Gegensatz zu der Familienzüchtung, wo die Nachkommenschaften zusammen frei abblühen und wo sich deshalb alles durchkreuzt, habe ich hier in aller Kürze auch praktisch erwähnen wollen, da ja diese Methode die Voraussetzung für die theoretischen Resultate ist. Dass man bei der praktischen Züchtung mit grösserem Erfolg mit EK und GK unter Separierung arbeiten kann als mit einer auch noch so sorgfältigen Familienzüchtung, ist ja selbstverständlich, weil die genische Differenzierung im ersten Fall bedeutend stärker wird. Denn bei der Familienzüchtung baut man die Nachkommenschaften verschiedenen Grades nebeneinander ganz offen. Zwar werden die besten, d. h. scheinbar besten, sorgfältig ausgelesen. Ob sie aber die besten sind, weiss man nicht, und nächstes Jahr wird genisch gutes und genisch schlechtes Material durcheinander gekreuzt. Jahr für Jahr wählt man das »morphologisch» beste, erhält aber ganz gewiss auch genisch schlechteres, denn vorauszusagen, was gut oder schlecht ist, kann kein Züchter. Nur eine effektive Separierung kann hier eine rationelle Scheidung erzielen. Denn wenn man auch, wie bei der Züchtung des berühmten Petkuserroggens, ganze Hektar von Nachkommenschaften aus hervorragenden Elitepflanzen aus den besten Familien baut und diese auf das sorgfältigste in bezug auf alle wichtigen Eigenschaften sortiert, so wird ja die Durchkreuzung im folgenden Jahr mindestens ebenso effektiv wie die Sortierung des vorigen. Diese fliessende Auslese ist keine Hochzucht, sondern eine Nivellierungszucht. Sie hält die Sorte auf der Ertragshöhe, aber kaum mehr. Aber dafür braucht man keine Züchtung, nur einen Schutz der Sorte gegen Vizinismus, was durch ihren Anbau auf grossen Arealen geschehen kann. Denn eine Population eines Fremdbestäubers ist ja als Variabilitätskreis konstant.

Um nun wieder zum Hauptproblem, der Inzucht, zurückzukehren, so könnte man vielleicht gegen meine Resultate einwenden wollen, dass mein Material in genotypischer Hinsicht vielleicht nicht genügend ausgeglichen gewesen sei. Hierauf kann ich antworten, dass der ganz überwiegende Teil der hier behandelten Nachkommenschaften aus Petkuserroggen stammt, weiter, dass sich die Resultate sehr schön nach der Stärke der Inzucht gradieren.

Es ist indessen möglich, meine Grade der Inzuchtdepression auch an einem genisch ausgeglichenen Material zu prüfen. Man kann nämlich an zwei Pflanzen mit genügend reicher Bestockung, die in einem

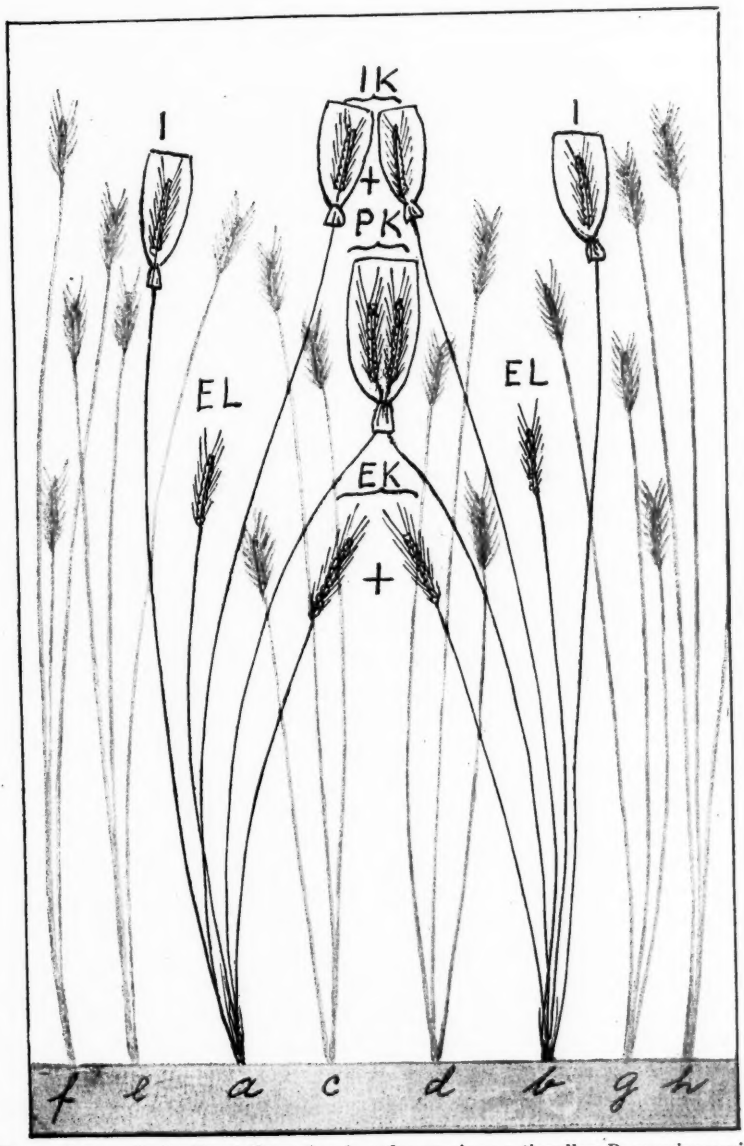

Fig. 1. Schematische Darstellung der Anordnung einer rationellen Depressionsserie.

Bestand stehen, gleichzeitig I, IK, PK, EL und EK erhalten. Man kann, wie ich dieses *experimentum crucis* nenne, eine *rationelle Depressionsserie* aufbauen.

In Fig. 1 habe ich eine derartige Serie übersichtlich veranschaulicht. In einem Bestand werden zwei Pflanzen als Inzuchteltern ausgewählt (*a* und *b*). Erstens werden Ährenisolierungen beider Pflanzen ausgeführt, von welchen die Körner separat ausgesät werden (I der Fig. 1). Zweitens werden von ebenfalls isolierten Ähren die Körner beider Pflanzen gemischt ausgesät (IK, das Zeichen + bei den Tüten gibt also die spätere Mischung der erhaltenen Körner an). Drittens werden zwei Ähren zusammen isoliert (PK). Viertens werden offen abgeblühte, von der Pollenwolke des Bestandes (von den Pflanzen *c*, *d*, *e*, *f*, *g*, *h*, u. s. w.) durchgekreuzte Ähren jeder Pflanze für sich zur Vermehrung ausgelesen (EL). Fünftens wird von jeder Pflanze eine frei gekreuzte Ähre genommen, diese Ähren aber zusammen entkörnt und die Körner also gemischt ausgesät (EK). Da man von jeder I und jeder EL zwei Nachkommenschaften hat, enthält eine vollständige rationelle Depressionsserie sieben Komponenten.

Mit diesen Serien konnte ich die durch die oben referierten Versuche festgestellten allgemeinen Inzuchtwirkungen auf eine ganz einwandfreie Basis stellen. Zuerst *schloss ich nämlich die genische Variabilität* so weit *aus*, wie es bei derartigen Versuchen überhaupt möglich ist, da ich bei *allen Inzuchtmethoden* von *denselben Eltern* ausging. Wichtig war indessen ferner, dass ich den zweiten Faktor, den man als ausschlaggebend für die Depression angegeben hat, nämlich die Homozygotie, so effektiv wie möglich eliminierte. Als Ausgangspopulation wählte ich deshalb eine Bestandkreuzung zwischen den schwedischen Sorten Stahlroggen und Sturmroggen, die sehr typendifferent sind. Sie wurden in wiederholten Reihen nebeneinander ausgesät und frei durchkreuzt. Aus der sehr heterozygoten Nachkommenschaft wurden die Elternpaare für die rationellen Depressionsserien (sie können kürzer RD-Serien genannt werden) ausgewählt. Die Möglichkeit, dass ich dann mit einem homozygoten Material gearbeitet habe, war natürlich ganz ausgeschlossen. *Ich ging also von einer weitgehenden Heterosis aus.*

Falls nun, da ich die genische Unterlage und die Heterosis so weit wie möglich konstant gemacht hatte, trotzdem eine graduelle Depression wie früher auftreten würde, so wäre diese nur als eine ganz besondere, allgemeine Inzuchtwirkung aufzufassen, die weder auf eine faktorielle Grundlage noch auf eine negative Heterosis zurückgeführt werden könnte. Die Antwort gibt Tabelle 2, in der die fünf bis jetzt durch-

geführten RD-Serien zusammengestellt sind. Da ich es als ganz unnötig erachtete, die I und IK überhaupt zu prüfen, habe ich mit nur vier Komponenten gearbeitet.

Die rationellen Depressionsserien bestätigen auffallend gut die Resultate meiner früheren Grossserien. Denn den höchsten Ertrag geben die EK, einen geringeren die EL und eine auffallend geringen die PK. *Daran, dass man es beim Roggen mit einer Depression zu tun hat, die nicht faktorieller oder homozygoter Natur ist, kann nicht gezweifelt werden.*

Als Vergleichssorte im Versuch ist wie gewöhnlich Petkuserroggen verwendet worden. Da indessen die EK hier aus einer Kreuzung Stahl × Sturm (oder, man schreibt besser Stahl + Sturm, wenn man auf eine Bestandkreuzung abzielt) abstammen, kann man natürlich

TABELLE 2. *Rationelle Depressionsserien aus einer Bestandkreuzung Stahlroggen + Sturmroggen.*

Typus der Inzucht	Körnerertrag, verglichen mit Petkuserroggen = 100					
	RDI	RDII	RDIII	RDIV	RDV	Durchschnittlich
PK	79,8	69,5	67,4	73,6	85,8	75,2
EL (erste)	88,1	82,8	80,4	83,7	96,1	86,2
EL (zweite)	86,2	77,2	86,8	94,2	89,3	86,7
EK	90,0	86,8	92,7	91,1	94,5	91,0

nicht erwarten, dass sie die Ertragsziffer 100 erreichen. Stahl gibt einen höheren Ertrag als Petkus, Sturm einen niedrigeren. Die Kreuzung als Population ist nur in einem einzigen Versuch geprüft worden und gab hier nur einen Ertrag von 87 %. Diese Zahl scheint nach dem durchschnittlichen Ertrag der EK etwas zu niedrig zu sein. Sie ist indessen für die Beurteilung der Inzucht von keiner grösseren Bedeutung. Da der Ertrag etwa 90 % zu betragen scheint, ist er in der Hinsicht von Interesse, dass er niedriger ausfällt als der beider Eltern und nicht zwischen diesen intermediär, da er ungefähr bei dem des Petkuserroggens liegen sollte. Obgleich also die Kreuzung Stahl + Sturm heterozygotischer ist als die Eltern, weist er doch einen geringeren Ertrag auf. Die Heterosiswirkung hat also eine Grenze, wo sie vom genischen Effekt gewisser Faktoren überflügelt wird. Das ist ja nach den allgemeinen Züchtungserfahrungen selbstverständlich. Denn sonst wäre ja nur »outbreeding», aber gar kein »inbreeding» möglich.

Die Schlussfolgerungen aus meinen Roggenstudien, die nicht hervordeduziert worden sind, sondern experimentell bei reiner Versuchsmethode sich ergeben haben, zeigen ein ganz anderes Bild von der Inzucht, als man es sich nach den Erfahrungen am Mais gebildet hat.

Zuerst fragt man also: Steht die Depression in Zusammenhang mit einer bei der Inzucht stets auftretenden stärkeren Homozygotie, und beruht also die Vitalität auf ein Restituieren der Heterozygotie? Ja, sagt man, denn man hat ja eine auffallende Stimulierung in F_1 von Inzuchtlinien gefunden. Nein, sage ich, denn bei grösster Heterozygotie hat man doch eine überaus kräftige Depression.

Diese Fragen bringen wir vielleicht am besten ins Reine, wenn wir die Resultate der PK betrachten. Die Mutterpflanzen der PK werden als die kräftigsten, am reichsten bestockten Pflanzen in Beständen ausgewählt, oder sie werden Linien entnommen, die schon durch ihren Ertrag in Feldversuchen sich als die besten gezeigt haben. Also kräftige Pflanzen von normaler Heterozygotie. Sie werden paargekreuzt. Die PK-Körner geben eine Nachkommenschaft, die in *vielen* Fällen ebenso heterozygot sein muss wie die Ausgangspopulation. Jedenfalls müssen die PK durchschnittlich den Ertrag der Elternpopulation geben. Aber nein. Der Ertrag ist in den Feldversuchen um 15 %, in den RD-Serien sogar um 25 % erniedrigt. *Diese gewaltige Depression tritt ohne jede Verminderung der Heterozygotie ein.* Einige PK sind allerdings weniger heterozygot als die Mutterpopulation, aber auch diese hat ja ihre Homozygoten. Durchschnittlich dürfte wohl die Heterozygotie der PK-Serie derjenigen der Ausgangspopulation ganz nahe kommen. Die Vitalität bleibt aber für die PK weit zurück.

Noch heterozygoter als die PK sind die EL. Denn sie sind ja die Nachkommenschaften *offen* abgeblühter Pflanzen. Aber auch hier ist eine durchschnittliche Depression von 10 % zu konstatieren. *Auch diese kann also keiner Homozygotwirkung zugeschrieben werden.*

Bei diesem Konstatieren ist es kaum eigentümlich, dass die Nachkommenschaften von Isolierungskreuzungen, obgleich F_1 stimuliert ist, in F_2 und folgenden Generationen, wenn die maximale Heterozygotie ausgeklungen ist, ein so überaus schlechtes Resultat in bezug auf den Ertrag zeigen. Denn hier wird wohl auch die Heterozygotie gewöhnlich nicht wiederhergestellt. Eine negative Heterosis ist hier nicht ausgeschlossen. Sonst könnte vielleicht die erhöhte Depression der IK-Serie auch der erzwungenen Selbstbefruchtung zugeschrieben werden.

Betrachtet man nun die Verwandtschaftsverhältnisse der Serien, so findet man, dass die IK, wo die Nachkommen Vollgeschwister aus

einelterlicher, hermaphroditer Zeugung sind, auch die grösste Depression zeigen. Bei den PK, wo die Nachkommen auch Vollgeschwister sind, aber aus von Anfang an diözischer Zeugung, ist die Depression auch auffallend stark. Bei den EL, wo die Nachkommen Halbgeschwister sind, ist die Depression vermindert, jedoch ganz deutlich. Bei den EK schliesslich, wo die Nachkommen zwei Halbgeschwisterkreise bilden, die sich frei durchkreuzen, ist die Depression ganz aufgehoben. Es sieht also so aus, als ob die Depression ganz parallel mit dem Grad der Verwandtschaft einherginge. Die alte Lehre von der Blutverwandtschaft als Ursache der Degeneration scheint demnach bestätigt worden zu sein.

Blutverwandtschaft ist indessen nur ein Ausdruck, der nichts besagt. Wir suchen aber keine mystische Umschreibung für den konstatierten Effekt der nicht homozygoten Depression, sondern eine mechanische Erklärung. Können wir denn die Parallelität zwischen Depression und Verwandtschaft ganz mechanisch veranschaulichen?

Es scheint mir so. Denn *diese eigentümliche Depression, die weder der genischen Rekombination noch dem Grad der Heterozygotie (Heterosis) folgt, scheint dagegen dem plasmatischen Effekt der Eltern in den Nachkommen zu folgen.*

Die I sind nämlich plasmatisch sehr eng konstituiert. Die Nachkommen haben alle ganz dasselbe Plasmon vom einzigen Elter erhalten. Wir können sie monoplasmonisch nennen. Die Depression ist ausserordentlich stark.

Die IK gehen von den zwei Plasmonen der I-Linien aus. Werden sie als manuelle Kreuzungen angelegt, und nicht reziprok ausgeführt, so erhalten sie ein mütterliches und ein vaterliches Plasmon. Da aber der Anteil des Plasmas bei der ♂-Gamete gering ist, so kommt dieser Typus der IK plasmatisch einer I nahe. Wird aber die IK reziprok oder durch Samenmischung von zwei I-Linien angelegt, so werden zwei ♀-Plasmone am Konstituieren beteiligt sein. Die IK wird also immer diplasmonisch angelegt, aber nicht immer mit gleichwertigen Plasmaanteilen. Ich habe diese beiden Typen nicht auseinander gehalten. Die diplasmonischen IK zusammengenommen weisen indessen eine sehr starke Depression, 25 %, in der Grossserie auf.

Die PK werden immer diplasmonisch konstituiert. Man sollte deshalb erwarten, dass sie, falls das Plasmon für die Depression eine ausschlaggebende Bedeutung hat, einen gleich verminderten Ertrag wie die IK zeigen sollten. Da aber einige IK nicht reziprok waren und deshalb, wie oben dargetan, wohl noch stärker deprimiert wurden, dürf-

ten die PK etwas höher liegen als die IK. Die Depression beträgt auch 15 % in der Grossserie. Für die rationellen Depressionsserien beträgt sie sogar 25 %, also gleich viel wie für die IK.

Die EL werden polyplasmonisch angelegt von einem grossen mütterlichen und zahlreichen kleinen väterlichen Plasmonen. Das Plasma, das der ♂-Gamete folgt, bildet ja nur eine winzige Hülle um den Kern der Spermazelle. Ein ♂-Plasmon hat man wohl früher ganz verneinen wollen, aber Spermazellen, und nicht nackte Spermakerne, sind ja nunmehr so zahlreich konstatiert, dass man ihr Vorkommen als eine normale Erscheinung annehmen kann. Man hat im ♂-Plasmon sogar ergrünende Chloroplasten konstatieren wollen. Die Plasmamenge, die eine männliche Sexualzelle umgibt, ist indessen quantitativ gegenüber der der Eizelle sehr gering. Auf den plasmonischen Effekt scheint diese quantitative Differenz kräftig einzuwirken. Denn beim Konstituieren der EL wirken wohl fast ebenso viele ♂-Plasmone mit wie die Ähre Körner hat, also 50—70. Wird die EL von mehreren Ähren gebildet, was ja bei kräftig bestockten Pflanzen geschehen kann, so erreichen die ♂-Plasmone mehrere Hunderte. Es tritt trotzdem eine Depression ein, die in der oben behandelten Grossserie von EL 10 % betrug.

Werden nun EK ausgeführt, so werden diese von zwei ♀-Plasmonen und zwei Kreisen von ♂-Plasmonen konstituiert. Die EK werden also von weiblicher Seite diplasmonisch, von männlicher sehr polyplasmonisch. Hier ist die Depression aufgehoben.

Da die EL Depression zeigten, nicht aber die EK, glaubte ich anfangs, dass eine einwurzelige (einmütterliche) Abstammung überhaupt depressiv wirkte. Da ich aber später fand, dass PK, die zweimütterlich angelegt werden, stark deprimiert werden, war diese Ansicht nicht aufrecht zu halten. Weiter zeigte sich auch, dass GK, die auch einwurzelig sind, keine Depression aufweisen. Sie sind jedoch nur EL, die durch die Gruppenkreuzung einer ausserordentlich starke polyplasmonische männliche Durchkreuzung ausgesetzt worden sind. Eine sehr stark erhöhte männliche Polyplasmonie kann also auch die Depression aufheben.

Aus allen diesen Tatsachen geht hervor, dass die quantitative plasmonische Verschiedenheit einer Nachkommenschaft für die Depression eine entscheidende Rolle spielt. Man könnte dieses Konstatieren auch so ausdrücken: *Homoplasmonie bedingt starke Depression. Heteroplasmonie ist in gewissem Grade für das Konstituieren einer normalen Vitalitätsbasis, auf der sich das genische Zusammenspiel*

geltend machen kann, unbedingt erforderlich. Mit dieser Annahme erhält die faktische, aber anfangs ganz ungereimte Depression nach Verwandtschaft, eine ebenso natürliche und mechanische Erklärung wie es eine faktoriell mendelsche sein würde. Sie wird aber *plasmonisch, nicht genisch.* Mit plasmonischen Erscheinungen in bezug auf die Vererbung sind wir ja schon bekannt, es kann deshalb für die Genetiker in dieser Erklärung nichts Befremdendes liegen.

Aber, sagt man natürlich, es ist deshalb nicht sicher, dass die Homoplasmonie die ganze Depression erklärt. Man hat vielleicht noch daneben eine deprimierende Homozygotie. Dies scheint mir nicht ausgeschlossen, ja sogar sehr wahrscheinlich. Denn die Depression nimmt in späteren Isolierungsgenerationen zu, obgleich die Homoplasmonie schon in I_1 erreicht wird. Ferner erhält man die grösste Stimulierung in F_1, wo man auch die maximale Heterozygotie hat. Dass man deshalb auch mit einer negativen und positiven Heterosis als Ursache der Depression und Stimulation rechnen muss, scheint mir sicher, auch für einen obligaten Fremdbestäuber sehr natürlich. Unter Heterosis verstehe ich dann ganz dasselbe wie ursprünglich SHULL, nämlich eine Stimulation, bzw. Depression, die durch den heterozygoten oder homozygoten *Zustand*, nicht aber von einer gewissen genischen Struktur verursacht wird.

Denn jeder Versuch, die *allgemeine* Depression, die ich bis jetzt hier abgehandelt habe, auf rein genischer Basis zu erklären, scheint mir daran zu scheitern, dass *jede* Pflanze, die man als Linie konstituiert, als EL also, sich als ertragärmer als die Elternpopulation zeigt. Aber diese kann ja offenbar nicht *nur* aus Minusvarianten bestehen. Das ist ja selbstverständlich. Und damit versagt jede rein genische Erklärung. Dass man, wie es JONES (1917) getan hat, in sehr geistreicher Weise veranschaulichen kann, wie man *in gewissen Fällen* durch Dominanz und Koppelung heterotische Phänomene erhalten kann, ändert hierin nichts.

Eine rein genische, d. h. faktorielle Depression hat man wohl, aber diese ist eine ganz *spezielle*, von letalen oder subletalen Faktoren verursacht. Reine Zwerglinien oder in gewissen Eigenschaften stark verkümmerte Formen habe ich auch beim Roggen erhalten, die oft wie die hochdeprimierten Inzuchtlinien (I_5 oder höher) aussehen. Sie bilden aber eine ganz besondere Gruppe, wo die Depression letaler Natur ist.

Man muss deshalb unterscheiden:

eine plasmonische Depression, die bei starker Homoplasmonie eintritt;
eine genomische Depression, die bei starker Homozygotie eintritt;
eine genische Depression, die durch letale Faktoren verursacht wird.

Die zwei ersten Gruppen rufen die allgemeine, deprimierende Inzuchtwirkung hervor, speziell die erstere, weil diese schon bei enger Paarung mit einem Schlag eintritt und eine starke Vitalitätsverminderung rein physiologisch verursacht, ohne eine besonders auffällige morphologische Deprimierung aufzuweisen. Die PK und EL sind nämlich oft von ziemlich normaler Entwicklung. Es ist kaum möglich aus ihrem Aussehen zu schliessen, dass sie einen 10—20 % geringeren Ertrag als die Ausgangspopulation geben werden. Dass diese Art der Depression deshalb, obgleich die praktischen Züchter zähe an ihr festhielten, während der letzten Jahrzehnte schlechtweg verneint worden ist, ist erklärlich. Es war ja auch bei dem klaren Unterordnen aller Eigenschaften dem genischen Vererbungsgang einem Genetiker ganz zuwider, die Enge der Verwandtschaft (die Blutverwandtschaft) als ausschlaggebend bei einer Erscheinung aufzufassen, die die Vitalität und den Ertrag einer Art betrifft, da man diese Eigenschaften bei den Selbstbefruchtern offenbar auf genische Faktoren hat zurückführen können. Mit einer plasmonischen Auffassung verschwindet auch die Mystik der Blutverwandtschaft. Die Tatsache einer nicht genischen und auch nicht genomischen allgemeinen Depression steht unter allen Umständen für den Roggen fest.

Die Frage liegt auf der Hand, ob die Resultate, die ich beim Roggen in bezug auf die Inzucht erhalten habe, sich für die Fremdbestäuber verallgemeinern lassen. Darüber kann ich nichts aussagen sondern nur hervorheben, dass Untersuchungen in dieser Richtung, jedenfalls für züchterisch bearbeitete Pflanzenarten, von fundamentaler Wichtigkeit sind. Verschiedene Fremdbestäuber scheinen sich indessen in bezug auf die Reaktion gegen erzwungene Autogamie ganz verschieden zu verhalten. Neben Roggen habe ich mit der allogamen *Oenothera Lamarckiana* lange Versuchsserien durchgeführt, und für gewisse Linien schon I_{19} gezüchtet. Trotz zwanzigjähriger erzwungener Autogamie sind diese Linien ebenso kräftig wie die Ausgangspflanzen und geben vollbesetzte Kapseln mit normal aussehenden und keimungsfähigen Samen. Nicht einmal I-Inzucht schadet dieser Art.

Sie ist im freien Bestand typisch allogam, aber selbstfertil, nicht wie der Roggen selbststeril. Möglich ist, dass gerade die selbststerilen und windpollinierenden Arten wie der Roggen für Inzucht besonders sensibel sind.

Die oben referierten rationellen Depressionsserien können für eine Entscheidung der Empfindlichkeit einer Art gegen Inzucht von grösster Bedeutung sein. *Die RD-Serien sind nämlich sehr gute Analysatoren für den Grad der Inzuchtsensibilität.* Durch jene erhält man nämlich eine sehr klare Übersicht über die Depression. Man braucht anfangs keine so langen und schwer durchführbaren Serien anzulegen, wie es meine Grossserien gewesen sind. Eine klare Antwort *in nuce* betreffs der Inzuchtsensibilität erhält man schon durch 5—10 RD-Serien. Danach weiss man auch, nach welchen Prinzipien der Inzucht das Material züchterisch handzuhaben ist.

Die hier behandelten Züchtungswege können in folgender Weise zusammengefasst werden.

A. *Isolierungszüchtung.*
   1. Individualisolierung (I).
   2. Isolierungskreuzung (IK).

B. *Separierungszüchtung.*
   1. Paarkreuzung (PK).
   2. Eliteauslese (EL).
   3. Elitekreuzung (EK).
   4. Gruppenkreuzung (GK).

C. *Familienzüchtung.*

D. *Massenzüchtung.*

Die genotypische Differenzierung des Züchtungsmaterials nimmt von oben nach unten ab, der Grad der Inzucht nimmt von unten nach oben zu. Wo die Möglichkeit höchster Differenzierung ohne gleichzeitige Depression liegt, können also die rationellen Depressionsserien entscheiden. Für den Roggen liegt dieser kritische Punkt niedrig, nämlich bei den Elitekreuzungen.

Für die freigebige Unterstützung, die mir C. F. LUNDSTRÖMS STIFTELSE während mehrerer Jahre gewährt hat, sage ich hier meinen ergebensten Dank.

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Vollständige Literaturangaben hinsichtlich dieses vielumschriebenen Gebietes sind in den oben angegebenen Arbeiten zu finden.

STUDIES IN DROSOPHILA MELANOGASTER WITH ATTACHED X:S

I. CROSSING-OVER VALUES. FREQUENCIES OF RECIPROCAL AND NON-RECIPROCAL EXCHANGES. CHROMATID INTERFERENCE

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I. INTRODUCTION.

THE first case of flies with attached *X* chromosomes was described by L. V. MORGAN (1922, 1925), and in her treatises a number of the fundamental genetic and cytological characters of these flies have been set forth. A thorough investigation regarding the crossing-over mechanism in this kind of flies was carried out by ANDERSON (1925), and later conditions in heterozygous attached *X* flies were studied by STURTEVANT (1931). In an essay on crossing-over in triploids with attached *X* chromosomes the present writer pointed out the desirability of systematic investigations on triploids as well as diploids with attached *X* chromosomes by identification of as many of the daughters as possible in regard to genotype (BONNIER, 1933). During the first part of 1932 identifications were made of 674 daughters of mothers of the constitution [1]

$$\frac{y \quad cv \quad ct \quad v \quad g \quad f}{+^{y} \quad +^{cv} \quad +^{ct} \quad +^{v} \quad +^{g} \quad +^{f}}$$

but as it proved desirable, later on, to study conditions in such animals also where the genes lie so close to each other that the coincidence may be assumed to be zero, in the latter part of 1934 identifications were

[1] In regard to those daughters, among whose offspring no forked daughters occurred, but where the number of offspring was so small that it could not be regarded as certain that the daughters were not heterozygous, tests have been carried out for another or more generations. In this manner the number of daughters classified as $\frac{+^{f}}{+^{f}}$, although they should rightly have been classified as heterozygous, has been reduced to a negligible minimum.

carried out of 550 wild-type daughters of mothers of the constitution

$$\frac{y \quad w^e \quad ec \quad rb \quad cv}{+^y \quad +^w \quad +^{ec} \quad +^{rb} \quad +^{cv}}$$

For various reasons the elaboration of these experiments could not be made immediately, and the publication has been delayed till now. Meanwhile the results of similar experiments have been published by BEADLE and EMERSON (1935).

II. NOMENCLATURE.

The nomenclature employed by different investigators varies a great deal. It would be highly desirable if an agreement on a uniform terminology could be reached. Below the nomenclature employed by BEADLE and EMERSON (1935) will in the main be followed, although some additional symbols will be introduced.

In the descriptions of the particular genes occurring in the experiments, we use the terms »left» and »right» in the generally accepted sense (as in the previous section). Thus, e. g., we write the gene *y* to the left of the gene *w* (certain exceptions are made in the treatment of homozygosis). But as all processes to be studied here proceed from the spindle-fibre attachment, i. e. from proximal to distal, we put the symbols for the proximal parts of the chromatids to the left of the symbols for their distal parts.

In order to distinguish between the two attached *X* chromosomes in the descriptions of the different phenomena, one of them will be called *A*, the other *B*. In meiosis one pair of attached chromatids will be symbolized A_1, B_1, the other pair A_2, B_2 (Fig. 1).

In regard to the exchange of chromosome parts, which is a result of crossing-over between the chromatids, we distinguish between *reciprocal* and *non-reciprocal* (*equational*) exchanges (Fig. 1).

A reciprocal exchange results from

crossing-over between A_1 and B_1, denoted by the symbol r_1,
or » » » A_2 » B_2, » » » » r_2.

A non-reciprocal exchange results from

crossing-over between A_1 and B_2, denoted by the symbol n_1,
or » » » A_2 » B_1, » » » » n_2.

Theoretically yet a third possibility exists, viz. that of crossing-over between sister chromatids (between A_1 and A_2 or between B_1 and B_2).

However, as it is now generally assumed that crossing-over of this kind does not occur, this possibility has not been included.

Quantitatively the classification has been made with regard to the total number of chiasmata which consequently corresponds to the total number of exchanges.

All the different types of exchanges can be described by com-

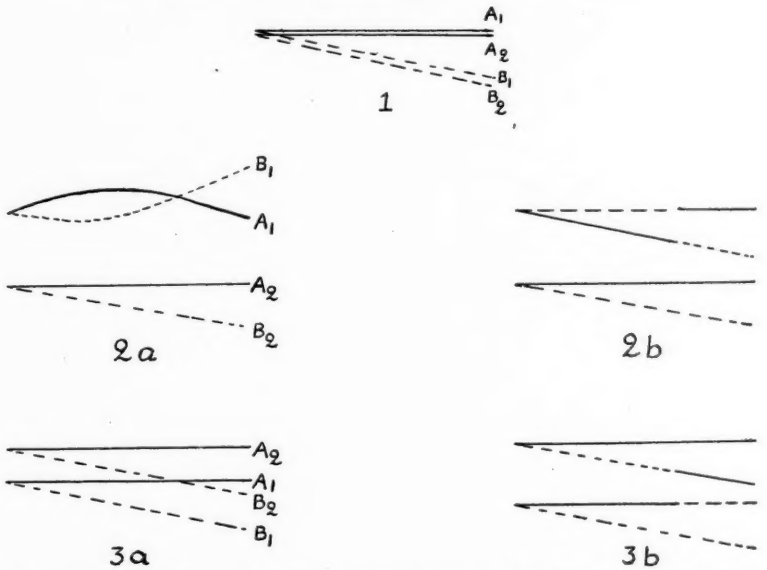


Fig. 1. Diagram of attached X chromosomes and single exchanges. — 1. The chromosomes split into chromatids A_1, A_2 and B_1, B_2. — 2. Reciprocal exchange (r_1): 2 a crossing-over, 2 b resulting chromosomes. — 3. Non-reciprocal exchange (n_1): 3 a crossing-over, 3 b resulting chromosomes.

binations of the symbols r and n (regard being paid to the order of the different points of crossing-over). For instance, r_1n_2 denotes a double exchange, resulting from a crossing-over between A_1 and B_1, followed distally by a crossing-over between A_2 and B_1.

III. SYNTHESIS OF THE MOTHERS.

As already stated the experiments described below were carried out in two different series. In the first series a number of genes, distributed along the whole length of the X chromosome, were fol-

lowed, in the other series the genes observed were crowded together within a smaller, more distally situated region of the chromosome. In both cases all the recessive mutant genes were situated in one of the two attached *X* chromosomes of the mothers (*B*), while the normal alleles were situated in the other *X* chromosome (*A*). The reason why all the mutants were brought together into one of the two *X* chromosomes was simply because in this way the experiments take less time and thus a larger material can be obtained. But in addition facts seem to indicate that in principle it is more correct to synthesize the mothers in such a manner that they have all mutants distributed in only one of the *X* chromosomes.

In most of the earlier investigations the mothers have been synthesized so as to get every other mutant gene into *A* and into *B*. This was done in accordance with the triploid investigations (BRIDGES and ANDERSON, 1925; REDFIELD, 1930), where the mutant genes were introduced into each of the three chromosomes. For a close study of the triploids evidently this manner of procedure is absolutely necessary, as landmarks are thus won within each of the chromosomes by which calculation of the frequencies of all the different cross-overs is made possible. In the case of diploids this reason does not apply. The different kinds of exchanges may be studied with the same ease when all the mutants are carried by one chromosome as when they are distributed on both, for there are only two alternatives for each location. But it is of importance in the case of diploids that the mothers are synthesized in such a manner that the source of error which consists in the varying viability of different phenotypes as far as possible is obviated. In ordinary diploids (i. e. without attached chromosomes) crossing-over studies are carried out by the aid of back crosses; consequently the mothers are synthesized in every possible manner so that each phenotype will appear both among the cross-overs and the non-cross-overs (balanced viability). This method is thus based on the correct view that the observed frequencies of different phenotypes should not unreservedly be accepted as indicating the corresponding zygotic frequencies.

In investigations on flies with attached *X* chromosomes classification of the phenotypes of the daughters alone does not tell us which crossing-over processes have occurred; instead it is necessary to analyze each individual daughter by starting a new culture so that each of the daughters will generate another batch of daughters. But daughters of certain phenotypes have a lower degree of viability than others, so

that they become under-represented, and besides they very often have lower fertility, and thus may not be analyzed with regard to their genotype. The consequence is that the daughters which have been identified in regard to their genotype by continued out-crossing, do not become distributed at random on the different phenotypes. In order to neutralize the effect of this circumstance, ANDERSON (1925) introduced a method (employed also by BEADLE and EMERSON, 1935), according to which the frequencies of the different genotypes within each group of identified mothers belonging to the same phenotype are multiplied by the percentage by which this phenotype occurs among the daughters. But this means that by this method the different penotype frequencies found among the daughters are accepted as measures of the zygotic frequencies of these phenotypes, which is exactly what we wish to avoid in the case of ordinary diploids.

It thus seems most appropriate to base the study of the exchange processes on the identification of daughters belonging to only *one* phenotype; the class chosen naturally is that consisting of wild-type daughters (and at the same time it is assumed that all the daughters of phenotypically wild type have a viability and fertility which is not influenced by the genotypical differences present). But this cannot be effected unless all the mutant genes belong to one of the *X* chromosomes, and the normal alleles to the other, which is the reason for synthesizing the mothers in the manner followed here. After every completed meiosis in the mother there are two unfertilized eggs, each containing two attached *X*:s; after fertilization with *Y*-spermatozoa these give rise to the zygotes out of which the daughters emerge. (One of these eggs of course is a non-functioning polar body, but as there is no reason to suppose that any selection of the *X* chromosomes on these cells takes place with regard to the genes carried by the *X*:s, they may, from the point of view of the frequencies to be expected, be regarded as two equivalent eggs.) For every individual locus there are 4 genes, one from each of the chromatids A_1, A_2, B_1, B_2 and when two of these have united in one egg, the two others must unite in the other. Thus, if one of the zygotes is a mutant homozygote $\frac{B_1}{B_2}$, the other must be a wild-gene homozygote $\frac{A_1}{A_2}$. But if one is a wild-type heterozygote $\frac{A_1}{B_1}$ $\left(\text{or } \frac{A_1}{B_2}\right)$, the other will also be a wild-type heterozygote $\frac{A_2}{B_2}\left(\text{or } \frac{A_2}{B_1}\right)$. Thus it must be a much more reliable method to replace the very

uncertain observed frequencies of mutant homozygotes by the wild-type homozygotes found.

Instead of using ANDERSON's (1925) method for the calculation of the number of mutant zygotes, we multiply by 2 all frequencies of wild-type daughters which are not heterozygous in regard to all the mutant genes. In our tables these frequencies are termed »corrected numbers». By this procedure, however, certain combinations of genes are altogether excluded, viz. those homozygous both in regard to mutants and wild-type genes. But it is exactly in this respect that an important difference manifests itself between this method of synthesizing the mothers and synthesizing them in each of the two attached *X* chromosomes. For when all the mutant genes in the mothers are situated in one and the same *X* chromosome, daughters homozygous for both mutant and normal genes are produced only after certain third rank exchanges, e. g. after $n_1 r_2 r_1$, or after still more complicated exchanges. On the other hand, if the mutant genes are situated in both of the *X* chromosomes, such daughters appear even after single exchanges. Consequently their frequency will be so small when the mothers have been synthesized in the manner em-

TABLE 1. *Daughters of females with attached X chromosomes of the constitution*

$$\frac{y\ \ cv\ \ ct\ \ v\ \ g\ \ f}{+^{y}\ +^{cv}\ +^{ct}\ +^{v}\ +^{g}\ +^{f}}$$

+	*y*	*y* *cv*	*y* *cv* *ct*	*y* *cv* *ct* *v*	*y* *cv* *ct* *v* *g*	*y* *cv* *ct* *v* *g* *f*	*cv*	*cv* *ct*	*cv* *ct* *v*	*cv* *ct* *v* *g*	*ct*	*ct* *v*	*ct* *v* *g*	*ct* *v* *g* *f*	*v*	*v* *g*	*v* *g* *f*	*g*	*g* *f*	*f*	Total
564	46	20	27	17	8	7	1	1	3	1	1	9	14	10	19	3	5	9	5	1	2	12	18	2	10	3	818
516	39	7	7	9	1	3	1	1	3	1	1	2	6	7	9	3	2	8	5	1	2	11	14	2	10	3	674

Total number of daughters .....

Number of daughters identified

TABLE 2. *Distribution of the genotypes of the 516 identified wild type daughters from Table 1.*

Genotype	Number	Corrected number	Genotype	Number	Corrected number
y cv ct v g f	137	137	y cv / v g f	1	2
cv ct v g f	27	54	y cv / g f	2	4
ct v g f	11	22	y cv / f	7	14
v g f	24	48	y cv	6	12
g f	21	42	y cv ct / v g f	31	31
f	16	32	y cv ct / g f	4	8
+ / +	4	8	y cv ct / f	7	14
y cv ct v f	1	2	y cv ct	7	14
y cv v g f	2	4	cv c t / v g f	2	4
y cv ct g f	1	2	y cv ct f / v g	1	1
y / cv ct v g f	40	40	y cv ct v / g f	18	18
y / ct v g f	2	4	y cv ct v / f	2	4
y / v g f	8	16	y cv ct v	12	24
y / g f	20	40	cv ct / g f	1	2
y / f	15	30	cv ct v / g f	4	8
y	10	20	ct v / g f	3	6
y v g f / cv ct	4	4	y cv ct v f / g	1	1
y g f / cv ct v	3	3	y cv ct v g / f	23	23
y f / cv ct v g	2	2	y cv ct v g	12	24
y / cv ct	1	2	cv ct v g / f	4	8
y / ct	1	2	ct v g / f	2	4
y / cv ct v g	1	2			
y cv / ct v g f	9	9			

Genotype	Number	Corrected number	Genotype	Number	Corrected number
$\frac{v\ g}{cv\ ct}\ \frac{}{f}$	1	2	$\frac{v\ g}{}$	2	4
	3	6	Total	516	763

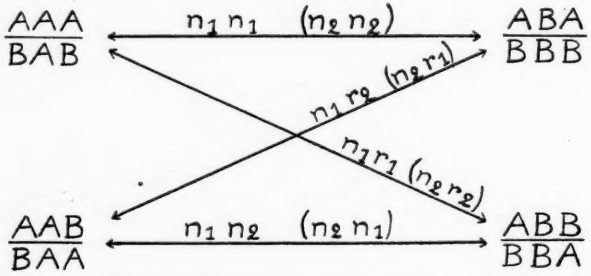
ployed here that omitting them must occasion a smaller error in the investigations in question than the error following from ANDERSON's method. The following comparisons in regard to these frequencies may be given:

Out of 79 non-wild type daughters (ANDERSON, 1925) 72 are homozygous for both wild type and mutant genes.

Out of 773 identified non-wild type daughters (BEADLE and EMERSON, 1935) 709 are homozygous for both wild type and mutant genes.

Out of 158 identified non-wild type daughters (Table 4) 6 are homozygous for both wild type and mutant genes.

It should perhaps be pointed out that in certain cases »corrected numbers» of the kind employed here, must be applied with a certain caution (cf. BEADLE and EMERSON, 1935, p. 198). Taking for instance the four zygotes



those to the left are homozygous with regard to one wild type gene (the middle one), and consequently correspond to one of the zygotes to the right which are mutant homozygous in regard to the middle gene. But it cannot be decided directly which one of the zygotes on the right corresponds to any one on the left. For instance, $\frac{AAA}{BAB}$ may be the product of an exchange n_1n_1 (or n_2n_2), and in that case cor-

TABLE 3. *Distribution of the genotypes of the 158 identified non-wild type daughters from Table 1.*

Phenotype	Genotype	Number	Phenotype	Genotype	Number
y	$\frac{y\ cv\ ct\ v\ g\ f}{y}$	32	*cv ct v g f*	$\frac{y\ cv\ ct\ v\ g\ f}{cv\ ct\ v\ g\ f}$	9
»	$\frac{y}{y}$	2	*ct*	$\frac{y\ cv\ ct}{ct\ v\ g\ f}$	3
»	$\frac{y\ cv\ ct}{y\quad v\ g\ f}$	2	*ct v*	$\frac{y\ cv\ ct\ v}{ct\ v\ g\ f}$	2
»	$\frac{y\ cv\ ct\ v\ g}{y\quad f}$	2	*ct v g*	$\frac{y\ cv\ ct\ v\ g}{ct\ v\ g\ f}$	8
»	$\frac{y\ cv\ ct\ v\ g}{y}$	1	*ct v g f*	$\frac{y\ cv\ ct\ v\ g\ f}{ct\ v\ g\ f}$	5
y cv	$\frac{y\ cv\ ct\ v\ g\ f}{y\ cv}$	6	*ct v f*	$\frac{y\ cv\ ct\ v\ f}{ct\ v\ g\ f}$	1
»	$\frac{y\ cv\ ct\ v\ g}{y\ cv}$	1	*v*	$\frac{y\ cv\ ct\ v\ g\ f}{v}$	2
y cv ct	$\frac{y\ cv\ ct\ v\ g\ f}{y\ cv\ ct}$	7	*v g*	$\frac{y\ cv\ ct\ v\ g}{v\ g\ f}$	7
y cv ct v	$\frac{y\ cv\ ct\ v\ g\ f}{y\ cv\ ct\ v}$	9	*v g*	$\frac{y\quad v\ g}{cv\ ct\ v\ g\ f}$	1
y cv ct v g	$\frac{y\ cv\ ct\ v\ g\ f}{y\ cv\ ct\ v\ g}$	1	*v g*	$\frac{cv\ ct\ v\ g}{v\ g\ f}$	3
y cv ct v g f	$\frac{y\ cv\ ct\ v\ g\ f}{y\ cv\ ct\ v\ g\ f}$	3	*v g f*	$\frac{y\ cv\ ct\ v\ g\ f}{v\ g\ f}$	13
y ct v g f	$\frac{y\ cv\ ct\ v\ g\ f}{y\quad ct\ v\ g\ f}$	1	*v g f*	$\frac{ct\ v\ g\ f}{v\ g\ f}$	1
y v g f	$\frac{y\ cv\ ct\ v\ g\ f}{y\quad v\ g\ f}$	1	*g*	$\frac{y\ cv\ ct\ v\ g}{g\ f}$	2
y g f	$\frac{y\ cv\ ct\ v\ g\ f}{y\quad g\ f}$	3	*g f*	$\frac{y\ cv\ ct\ v\ g\ f}{g\ f}$	8
y f	$\frac{y\ cv\ ct\ v\ g\ f}{y\quad f}$	1	*g f*	$\frac{y\ cv\quad g\ f}{ct\ v\ g\ f}$	1
cv	$\frac{cv\ ct\ v\ g\ f}{y\ cv}$	1	*g f*	$\frac{g\ f}{y\ cv\ ct\ v\ g\ f}$	1
cv ct	$\frac{y\ cv\ ct}{cv\ ct\ v\ g\ f}$	2	*f*	$\frac{y\ cv\ ct\ v\ g\ f}{f}$	2
cv ct v	$\frac{y\ cv\ ct\ v}{cv\ ct\ v\ g\ f}$	6	*f*	$\frac{y\quad f}{cv\ ct\ v\ g\ f}$	1
cv ct v g	$\frac{y\ cv\ ct\ v\ g}{cv\ ct\ v\ g\ f}$	7		Total	158

TABLE 4. *Daughters of females with attached X chromosomes of the constitution*

$$\frac{y\ w^e\ ec\ rb\ cv}{+^y\ +^w\ +^{ec}\ +^{rb}\ +^{cv}}$$

+	*y*	*y* *w*^{e}	*y* *w*^{e} *ec*	*y* *w*^{e} *ec* *rb*	*y* *w*^{e} *ec* *rb* *cv*	*w*^{e} *ec*	*w*^{e} *ec* *rb*	*w*^{e} *ec* *rb* *cv*	*ec*	*ec* *rb* *cv*	*rb* *cv*	*cv*	Total
859	6	11	8	21	85	1	1	1	1	5	1	10	1010

Identified: 550 + daughters

TABLE 5. *Distribution of the genotypes of the 550 identified wild type daughters from Table 4.*

Genotype	Number	Corrected number	Genotype	Number	Corrected number
$\frac{y\ w^e\ ec\ rb\ cv}{}$	322	322	$\frac{y\ w^e}{ec\ rb\ cv}$	7	7
$\frac{w^e\ ec\ rb\ cv}{}$	7	14	$\frac{y\ w^e}{rb\ cv}$	1	2
$\frac{ec\ rb\ cv}{}$	16	32	$\frac{y\ w^e}{cv}$	2	4
$\frac{rb\ cv}{}$	6	12	$\frac{y\ w^e}{}$	14	28
$\frac{cv}{}$	25	50	$\frac{y\ w^e\ ec}{rb\ cv}$	6	6
$\frac{+}{+}$	101	202	$\frac{y\ w^e\ ec}{}$	5	10
$\frac{y}{w^e\ ec\ rb\ cv}$	4	4	$\frac{y\ w^e\ ec\ rb}{cv}$	14	14
$\frac{y}{cv}$	1	2	$\frac{y\ w^e\ ec\ rb}{}$	16	32
$\frac{y}{w^e\ ec}$	2	4	Total	550	747
	1	2			

responds to $\frac{ABA}{BBB}$. But $\frac{AAA}{BAB}$ may also be the product of an exchange n_1r_1 (or n_2r_2), and in that case corresponds to $\frac{ABB}{BBA}$. These correspondences are indicated in the diagram p. 264. So long as only the relations are studied between two consecutive loci, so close to each

other that only simple exchanges between them are to be reckoned with (*r* or *n*), our »corrected numbers» can be freely applied.

The results of the matings are to be found in Tables 1—5. As already stated, in this investigation no analysis has been based on the identification of non-wild type daughters, but a good deal of work has been expended on the identification of non-wild type daughters; for the sake of completeness the results from the first series are given in Table 3.

IV. CROSSING-OVER PERCENTAGES.

For common diploids (i. e. without attached chromosomes) the recombination percentage is employed as crossing-over percentage, provided that the region studied is so short that as a result of the coincidence value, it is unnecessary to reckon with cross-overs of a higher rank than the first. For cases with attached chromosomes, the crossing-over percentage may also be calculated as the quotient between the number of recombinations found and the whole number of chromosomes. For regions of not too great a length a sufficiently good approximation is obtained in that way. When the regions are too long, the method will give unduly low values. Hence in any comparison between the values found by us and the standard values it must be borne in mind that the values found must be regarded as minimum values (Table 6). It its therefore particularly noteworthy that in all regions (excepting the region *cv—ct*) the crossing-over percentages found are considerably *higher* than the standard values. The explanation might be that the strains used in the experiments are characterized by a particularly high crossing-over frequency. As a matter of fact, it seems probable that to a certain degree the extremely high percentage within the region *y—cv* must be explained in this manner. But this does not seem to be the whole explanation. It seems probable that flies with attached *X* chromosomes really do have a crossing-over frequency higher than those with free *X* chromosomes. That this has not been apparent from experiments carried out by other investigators is probably due to the fact that they have synthesized the mothers with mutant genes in both the *X* chromosomes. Thus the lower viability of the mutant types has reduced the frequency of cross-overs more than that of non-cross-overs. As regards the work of BEADLE and EMERSON (1935) in particular they obtained crossing-over values in agreement with the standard values in the distal part of the chromosome, while already proximally to vermilion they found a reduction

TABLE 6. *Percentages of crossing-over.*

	Region								
	Table 5				Table 2				
	y—w	*w—ec*	*ec—rb*	*rb—cv*	*y—cv*	*cv—ct*	*ct—v*	*v—g*	*g—f*
	2,0	5,6	2,5	7,8	19,7	6,0	13,5	13,1	13,6
Standard	1,5	4,0	2,0	6,2	13,7	6,3	13,0	11,4	12,3

of these values (within the interval from *cv* to *v* they obtained somewhat higher crossing-over values than the standard values). The reason for this reduction — apart from the lower viability of the mutant types — is to be found in the circumstance that they worked with a strain with particularly low crossing-over frequency within the most proximal part of the chromosome. This fact is pointed out by the authors themselves.

V. THE RELATIVE FREQUENCY OF RECIPROCAL AND OF NON-RECIPROCAL SINGLE EXCHANGES.

When studying a certain region it is possible to pick out all the flies which are heterozygous for the gene forming its proximal end. Of these we take under observation the following two groups

$$\frac{AB}{BA} \qquad \frac{AA}{BA}$$

denoting the numbers found (»corrected numbers») h and k respectively. (Heterozygosis in the proximal end may be either reciprocal, $\frac{A_1}{B_1}$ and $\frac{A_2}{B_2}$, or non-reciprocal, $\frac{A_1}{B_2}$ and $\frac{A_2}{B_1}$. But if this latter form is present, at least a third rank exchange must have taken place proximally to the distal end of the region, and the frequency of such an occurrence is so low that it can be omitted.) If the region is sufficiently short so that only single exchanges within it have to be reckoned with, half of all the exchanges r will give rise to the first group, and all exchanges n to the second group. Thus if the probability is the same for reciprocal and non-reciprocal exchanges, we get

$$2h = k.$$

The easiest way to ascertain whether this equation really applies is by means of the χ^2 method. This test is given in Tables 7 and 8.

Here a separation of the results has been made with regard to the two series of experiments, and it is especially to the short regions that the above relation should apply. We find that for two of the short regions (Table 7) there is statistical certainty that the non-reciprocal

TABLE 7. *Test of equality of reciprocal and non-reciprocal exchanges (from crosses with females heterozygous with respect to y, w^e, ec, rb and cv).*

	Region								Total
	y—w^e		*w^e—ec*		*ec—rb*		*rb—cv*		
	2h	*k*	*2h*	*k*	*2h*	*k*	*2h*	*k*	
	8	16	14	32	12	14	28	56	
χ^2	2,6666		7,0434		0,1538		9,3334		19,1972
Degrees of freedom	1		1		1		1		4
P <	0,20		0,01		0,50		0,01		0,01

TABLE 8. *Test of equality of reciprocal and non-reciprocal exchanges (from crosses with females heterozygous with respect to y, cv, ct, v, g and f).*

	Region										Total
	y—cv		*cv—ct*		*ct—v*		*v—g*		*g—f*		
	2h	*k*	*2h*	*k*	*2h*	*k*	*2h*	*k*	*2h*	*k*	
	106	82	18	38	80	76	72	98	82	96	
χ^2	3,0638		7,1428		0,1026		3,9764		1,1012		15,3868
Degrees of freedom	1		1		1		1		1		5
P <	0,10		0,01		0,80		0,05		0,30		0,01

exchanges are more frequent than the reciprocal ($P < 0{,}01$), and taking all regions in Table 7 together it will also be found that there is statistical certainty ($P < 0{,}01$) for a surplus of non-reciprocal exchanges. Besides, the variation in the proportion of reciprocal and non-reciprocal exchanges between these short regions is not so large that any inhomogeneity need be assumed; this fact was pointed out to me by

Dr. MATHER. As a matter of fact a homogeneity calculation from Table 7 gives the values: $\chi^2 = 1{,}9655$, degrees of freedom $= 3$, $0{,}5 < P < 0{,}7$. For the longer regions in Table 8 it will also be found that for the total there is statistical certainty for a higher frequency of the non-reciprocal than of the reciprocal exchanges. But this certainty originates from the region *cv—ct* only, because here the regions are not homogeneous. ($\chi^2 = 14{,}0461$, degrees of freedom $= 4$, $P < 0{,}01$). Within two of the regions there is a surplus of reciprocal exchanges, and in the region *y—cv* in particular this surplus is of 90 % significance. This is remarkable because precisely this region includes all the short regions from Table 7. The conclusion would seem to be justified that within certain *Drosophila* strains and within certain regions of the *X* chromosome there is a stronger tendency for non-reciprocal than for reciprocal exchanges to occur.

VI. FREQUENCIES OF HOMOZYGOSIS AND PROOF OF CHROMATID INTERFERENCE.

The frequency of homozygosis in different loci has been the subject of several investigations (L. V. MORGAN, 1925; ANDERSON, 1925; STURTEVANT, 1931; RHOADES, 1931; BEADLE and EMERSON, 1935). On the assumption that all exchanges of the same rank are equally frequent the percentage of homozygosis in a given locus may be calculated from the number of single, double etc. crossing-overs proximally to this locus. Calculations on this problem have been carried out by SAX (1932), BELLING (1933), MATHER (1935), BEADLE and EMERSON (1935) and WEINSTEIN (1936). In the same manner it is also possible to calculate the frequencies of reciprocal heterozygotes $\left(\frac{A_1}{B_1} \text{ or } \frac{A_2}{B_2}\right)$ and of non-reciprocal heterozygotes $\left(\frac{A_1}{B_2} \text{ or } \frac{A_2}{B_1}\right)$. In order to do this it is only necessary to keep the position clear, that if we have two loci between which there is *one* chiasma, the following must apply:

Nature of the zygote in the proximal locus	Nature of exchanges	Nature of the zygote in the distal locus
Homozygote	*r*	Non-reciprocal heterozygote
»	*n*	Reciprocal »
Reciprocal heterozygote	*r*	» »
» »	*n*	Homozygote
Non-reciprocal heterozygote	*r*	»
» »	*n*	Non-reciprocal heterozygote

It will then be found that reckoning with at most 4 chiasmata proximally to the locus under consideration

the frequency of	homozygotes	$= m_1 - m_2 + 3\,m_3 - 5\,m_4$
» »	reciprocal heterozygotes	$= m_0 + m_2 - m_3 + 3\,m_4$
» »	non-reciprocal »	$= m_2 - m_3 + 3\,m_4$

Here m_0, m_1 . . . m_4 denote the number of single strands found which are non-cross-overs, single cross-overs . . . quadruple cross-overs respectively. (The above frequency of homozygotes includes both kinds. In order to find the frequency of only one kind of homozygotes — e. g. the recessive, which is mostly in question — we have to divide by 2.)

Possibly the division of the heterozygotes into the two classes will not be found to be of any general use. But the calculation made shows that — under the conditions assumed — the frequency of non-reciprocal heterozygotes is so low that they may often be omitted. Whether the probability is equally great for the occurrence of the different exchanges of the same rank or not, a non-reciprocal heterozygote requires for its occurrence the existence of at least two chiasmata proximally to the locus studied. At the spindle fibre attachment — i. e. at the proximal terminal points of the chromosome — we always find reciprocal heterozygosis, and the above scheme shows that if a locus with non-reciprocal heterozygosis is to be reached from here, there must be at least one double exchange *nr*. Thus, if a region is studied within which one exchange has occurred, the proximal end of which is non-reciprocally heterozygous, there must be at least 3 exchanges proximally to its distal end. Assuming that the frequency of 3 (or more) exchanges is very low, consequently our method, as employed in the previous section, of comparing the frequency of reciprocal and non-reciprocal exchanges in the different regions is hereby justified. That in reality the frequency of third rank exchanges is very low seems to be evident, having regard to the fact that in our first series of experiments we have not found a single triple cross-over chromosome (Table 9). BEADLE and EMERSON (1935) did not find more than 0,26 % (»proportional percentages») of triple cross-overs, and no quadruple cross-overs.

In Table 9 will be found the frequencies (»corrected numbers») of single strands with regard to their nature proximally to the genes occurring in the first series of experiments. (The second series, the most proximal gene of which is *cv*, is of less interest in this connection, and is therefore not discussed here.) However, the reciprocal exchanges

occurring proximally to forked do not manifest themselves. Therefore, the unknown number of reciprocal exchanges proximally to forked must be added to the number of single cross-overs, and subtracted from the number of non-cross-overs. In the table this number has been denoted by x. BEADLE and EMERSON (1935) put this number x equal

TABLE 9. *Frequencies of single strands according to their nature proximal to the loci studied.* (x = *non-detectable reciprocals proximal to forked.*)

	y	*cv*	*ct*	*v*	*g*	*f*
m_0 = Non cross-overs	$488-x$	$732-x$	$808-x$	$1004-x$	$1200-x$	$1408-x$
m_1 = Single »	$952+x$	$764+x$	$704+x$	$518+x$	$326+x$	$118+x$
m_2 = Double »	86	30	14	4	—	—

to the number of single strands found, which are cross-overs proximally to the most proximal gene, in their case »carnation». In our case, accordingly, x would be 118, but as we have shown that the frequencies of reciprocal and non-reciprocal exchanges are not necessarily the same, we do not introduce any definite figure for x. For our conclusions the value of x is of no importance.

TABLE 10. *Percentage of homozygosis.*

$$z = \frac{x}{3052}.$$

	y	*cv*	*ct*	*v*	*g*	*f*
Expected.....	$28{,}4+z$	$24{,}0+z$	$22{,}6+z$	$16{,}8+z$	$10{,}7+z$	$3{,}9+z$
Found	16,4	18,4	18,2	16,5	12,1	7,7
Difference	$12{,}0+z$	$5{,}6+z$	$4{,}4+z$	$0{,}3+z$	$-1{,}4+z$	$-3{,}8+z$

Calculations of the *expected* frequencies of homozygosis, based on the frequencies found and the formula for homozygosis, have as a rule shown very good agreement with the frequencies of homozygosis actually found (BEADLE and EMERSON, 1935). Naturally this has been regarded as lending support to the view that all exchanges of the same rank are equally probable. In our case, however, we get no such agreement, but a difference increasing from proximal to distal, with a

regression which is not far from linear (Table 10 and Fig. 2). It should be observed that the »found» percentages in Table 10 are calculated by means of »corrected numbers» from Table 2, and consequently represent in reality the percentage of daughters homozygous in regard to the wild allele. In the locus for forked we found an unusually high percentage: 7,7. The following values were found by L. V. MORGAN (1925), ANDERSON (1925), STURTEVANT (1931), RHOADES (1931) and BEADLE and EMERSON (1935): 5,1, 5,2, 5,1, 4,9 and 2,5. However, if we compare the percentages of homozygosis found, according to »corrected numbers» and according to the number of mutants found (Tables 1

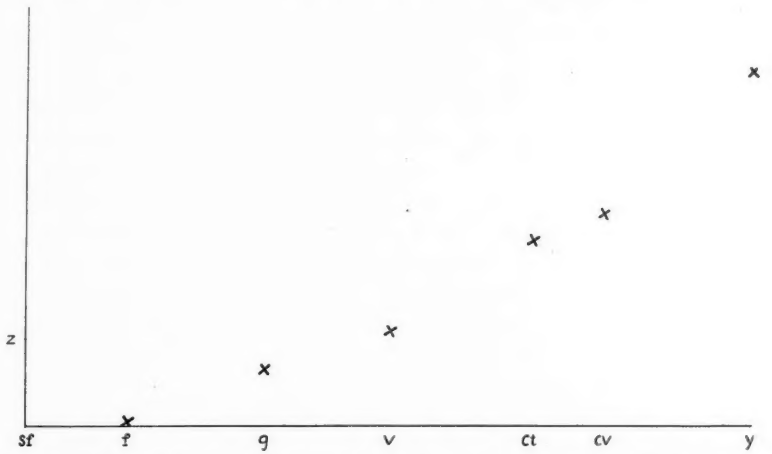


Fig. 2. Diagram showing differences (ordinates) between expected and found percentages of homozygosis (based on the hypothesis of no chromatid interference) as functions of map distances (abscissas).

and 4), we find that the former are the higher, excepting only forked, which occurs with 8,4 % in Table 1.

The probable reason why the difference between expected and found values increases, as we pass from proximal to distal, is that the assumptions for the formula, by which the »expected» frequencies are calculated, do not hold in our case. Part of the difference may be explained if the exchanges *r* are more frequent than the exchanges *n*, but we know from the calculations in the previous section that no notable contribution to an equalisation between expected and found values is obtained in this manner (v. infra). An explanation which seems more plausible is that the exchanges of the second rank are not

equally frequent. Part of these exchanges lead to the occurrence of only single cross-overs, others to the occurrence of non-cross-overs, single cross-overs and double cross-overs, and others, finally, to the occurrence of non-cross-overs and double cross-overs. With regard to this circumstance the 16 possible exchanges of the second rank are distributed as follows:

1. Exchanges resulting in single cross-overs only r_1r_2, r_2r_1, n_1n_2, n_2n_1
2. Exchanges resulting in non-cross-overs, single cross-overs and double cross-overs in the proportion 1 : 2 : 1 all *rn* and all *nr*
3. Exchanges resulting in non-cross-overs and double cross-overs in the proportion 1 : 1 r_1r_1, r_2r_2, n_1n_1, n_2n_2

All 4 chromatids take part in the exchanges within group 1, but each separate chromatid has only *one* point of crossing-over (=4-strand doubles). 3 chromatids take part in the exchanges in group 2, one of them having two points of crossing-over with each of the other two, the latter thus having only *one* point of crossing-over (= 3-strand doubles). Only 2 chromatids, finally, take part in the exchanges in group 3, both showing 2 points of crossing-over, which they have in common (= 2-strand doubles).

According to Table 9, we have found no double cross-over strands proximal to garnet, but according to Table 10 the difference between expected (on the assumption of equal frequencies of all kinds of exchanges of one and the same rank, i. e. on the assumption of no *chromatid interference*) and found percentages of homozygosis is greater in the region between garnet and forked than proximal to forked. This must probably be due to one of the following two reasons:

1) Among the single exchanges in the two regions garnet to forked, and forked to spindle attachment there is a greater proportion of reciprocals in the former region than in the latter.

2) There actually occur double exchanges in the region garnet to forked, but these exchanges do not result in any double cross-over strands.

Of these two assumptions 1) is not very probable, since Table 8 shows that there has been an excess — though a slight one — of *non*-reciprocal exchanges in the region garnet to forked. We are therefore more or less compelled to take assumption 2) for granted. But as the only kind of double exchanges, which do not result in any double cross-

over strands, are the 4-strand doubles, assumption 2) is equivalent to the following assumption: In a region proximal to a locus, which is itself situated as proximal as garnet is, there is a strong chromatid interference, allowing 4-strand double exchanges to occur but making the occurrence of 3- and 2-strand double exchanges impossible.

Let us now pass on to the regions distal to garnet. In order to do this we suppose that there are equal frequencies of reciprocal and non-reciprocal single exchanges. As a matter of fact we know that this may not always be the case but we shall later on discuss in what direction such an assumption influences our conclusions (v. infra). This assumption includes inter alia that the number z from Table 10 is equal to 3,8. Further, we suppose that there are *unequal* frequencies of the different kinds of double exchanges in such a way that the proportions of 2-strand, 3-strand and 4-strand double exchanges are as $c_2 : c_3 : c_4$. (In conformity with the assumption made concerning the single exchanges, we suppose, however, that *within* each of these three groups of double exchanges there are *equal* frequencies.) Therefore

$$c_2 + c_3 + c_4 = 1.$$

(In the case of no chromatid interference, in which case the »differences» from Table 10 should equal z, we have $c_2 = c_4 = {}^1/_4$, $c_3 = {}^1/_2$.) On these assumptions and supposing that there is no need to reckon with exchanges of a higher rank than the second, it is possible to calculate the expected percentages of homozygosis of the different mutant genes in exactly the same way as the formula p. 271 was derived. It may thus be shown that this percentage is:

$$\frac{m_1}{2} - \frac{2m_2c_4}{c_3 + 2c_2}$$

where m_1 and m_2, as before, denote the number of actually found single and double cross-over strands (expressed as percentages). If we express the »difference» from Table 10 as the proportion of the corresponding value of m_2 from Table 9, and denote this proportion a (Table 11) we must have

$$\frac{m_1}{2} - \frac{m_2}{2} - \frac{m_1}{2} + \frac{2m_2c_4}{c_3 + 2c_2} = a\, m_2$$

or

$$c_4 = (c_3 + 2c_2)\frac{2a + 1}{4}$$

$$\text{or again if } b = \frac{2a + 1}{4} \text{ (Table 11)}$$

$$c_4 = b(c_3 + 2c_2)$$

If both the proportions c_3 and c_2 had values corresponding to no chromatid interference, i. e. $c_3 = 0{,}5$ and $c_2 = 0{,}25$, the value of c_4 would equal b. But as $c_2 + c_3 + c_4 = 1$ this means that in such a case $b = 0{,}25$. Glancing at Table 11 we find that for all loci $b > 0{,}25$, and the conclusions to be drawn are the following: proximal to garnet $c_2 = c_3 = 0$; proximal to the other loci c_2 or c_3 or both have smaller values than they would have, if there was no chromatid interference, and in such a way that $c_3 + 2\,c_2 < 1$. But as b is diminishing when going from proximal to distal $c_3 + 2\,c_2$ may at the same time approach unity, or in other words: the further distal the locus concerned is situated the smaller is the influence of the chromatid interference.

TABLE 11.

	y	*cv*	*ct*	*v*
a = »difference» from Table 10 as proportion of m_2 from Table 9	0,1837	0,3133	0,5857	1,0000
$b = \frac{2a + 1}{4}$	0,3419	0,4067	0,5429	0,7500

We have arrived at this conclusion under the assumption that there are equal frequencies of reciprocal and non-reciprocal *single* exchanges. In the regions, however, where an inequality seems to be certain (cross-veinless to cut and possibly vermilion to garnet; Table 8) there is an excess of *non*-reciprocal exchanges, and this would only accentuate the inequality $c_3 + 2\,c_2 < 1$.

We have thus found that there is a higher proportion of 4-strand doubles than of 3- and 2-strand doubles. The relation *between* 3- and 2-strand doubles cannot be found in this way. But if the supposition of the phenomenon of interference as being a consequence of the rigidity of the chromosomes (which was made before it was known that crossing-over takes place at a 4-strand stage) is correct also for the chromatids, it seems necessary to conclude that not only are the 4-strand doubles more probable to occur than the 3-strand doubles (which is in conformity to what we have found actually to be the case) but that

also the 3-strand doubles are more probable to occur than the 2-strand doubles.

Our conclusions are of course valid only for crosses with strains of the same kinds as ours. For other strains the chromatid interference may be more difficult or even impossible to detect — if there is any at all (cf. BEADLE and EMERSON, 1935).

SUMMARY.

1. In this paper the results of crosses with flies having attached *X* chromosomes are given. These crosses have been carried out in two series, one with females of the constitution

$$\frac{y \quad cv \quad ct \quad v \quad g \quad f}{+^{y} \; +^{cv} \; +^{ct} \; +^{v} \; +^{g} \; +^{f}}$$

the other with females of the constitution

$$\frac{y \quad w^{e} \quad ec \quad rb \quad cv}{+^{y} \; +^{w} \; +^{ec} \; +^{rb} \; +^{cv}}$$

2. These females, accordingly, had all their mutant genes situated in one and the same chromosome. The desirability of the synthesis of the females being made in this manner has been pointed out, as in that way only, does it become possible to base all the calculations of the crossing-over percentage on the identification of wild-type daughters alone. In the cases where the calculation has to be based on the identification of non-wild type daughters also, the risk is present of obtaining too low percentages of crossing-over, owing to the relatively lower viability of the mutant types.

3. The crossing-over percentage for all the regions examined (with the exception of region *cv-ct*) has proved to be greater than the standard values. This circumstance may be partly due to the fact that the strains employed were characterized by unusually high crossing-over percentages. It may also be partly due to this percentage being actually higher in animals with attached *X* chromosomes than in animals with free *X* chromosomes.

4. Comparisons have been made of the relative frequency of reciprocal and non-reciprocal single exchanges. These show that at least in certain strains and in certain regions, a difference in these frequencies is present, as a rule in favour of the non-reciprocal exchanges.

5. On comparing the homozygosis percentages found with those calculated on the basis of the presumption that all different kinds of

exchanges of one and the same rank have the same frequency, the experiments show great deviations. The regression of these deviations on the genetic distance from the proximal terminal point of the chromosome is not far from linear.

6) This discrepancy is shown most probably to be a consequence of a chromatid interference, making the 4-strand double exchanges more frequent than the 3- and 2-strand double exchanges. The influence of this chromatid interference is, however, smaller the further distally the locus concerned is situated. It is pointed out that it is also probable that 3-strand double exchanges are more frequent than the 2-strand double exchanges. This situation seems also to be necessary if the chromosome rigidity, which has for a long time been supposed to be the cause of chromosome interference, also holds good for the chromatids.

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ZYTOLOGISCHE UND MORPHOLOGISCHE VALERIANA-UNTERSUCHUNGEN

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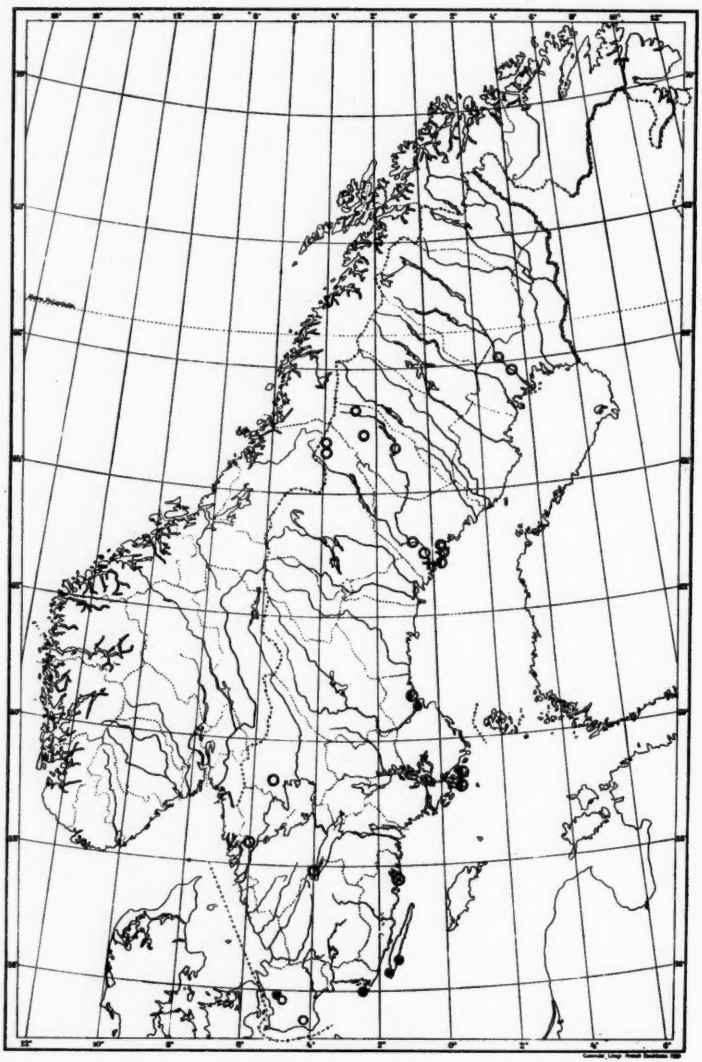
(Vorläufige Mitteilung)

SEIT ASPLUND im Jahre 1920 seine Arbeit über die Gattung *Valeriana* veröffentlichte, sind seine Untersuchungen von anderen Forschern, MEURMAN (1925 a, 1925 b, 1931), SENJANINOVA (1927) und PROTASSENJA (1930), fortgesetzt worden. Die von ASPLUND für *V. officinalis* angegebene haploide Chromosomenzahl 32 wurde von MEURMAN (1925 b) auf 28 abgeändert, der gleichzeitig seiner Auffassung Ausdruck gibt, dass *V. officinalis* mit der von PLEIJEL im Jahre 1925 beschriebenen neuen Art *V. salina* identisch ist.

Innerhalb der Gattung *Valeriana* gibt es zwei Chromosomenzahlserien; die eine mit der Grundzahl 8, zu der z. B. *V. montana* und *V. dioica* gehören, und die andere mit der Grundzahl 7. Zu der letzteren Serie rechnet man u. a. *V. officinalis*, *excelsa* und *nitida*. Sämtliche Arten mit Ausnahme von *V. montana* und *nitida* kommen in Skandinavien vor.

Um die Chromosomenzahl der schwedischen *Valeriana*-Arten feststellen zu können, wurde während des Sommers 1936 teils wildwachsendes Material von verschiedenen Teilen des Landes, teils lebendes Material von einigen Botanischen Gärten gesammelt. Das Material wurde dann zum Zwecke weiteren Anbaus in Svalöf gesammelt, wo es auch bearbeitet wurde. Die von verschiedenen Fundorten stammenden Arten, die einer vorläufigen Untersuchung unterworfen wurden, sind auf der Karte eingezeichnet. Von meinen Untersuchungen, die noch nicht zu Ende geführt sind, teile ich im folgenden die wichtigsten Ergebnisse mit.

V. officinalis hat nach PLEIJEL (1925) eine Verbreitung, die sich vom südlichen Schonen bis ungefähr zum 60. Breitegrad erstreckt. Diese Art ist hauptsächlich an die Küste, und zwar an die östliche, gebunden und fehlt deshalb fast ganz an der schwedischen Westküste. In Norwegen findet man sie an der Küste bis hinauf zum Trondheimsfjord und in Finnland an einigen Orten am Finnischen Meerbusen. Das Charakteristischste für diese Art ist die Zahl der Blättchen, die



Skandinavien. — ● *Valeriana officinalis* L. — ⊗ *V. baltica* PLEIJEL. — ◐ *V. salina* PLEIJEL.. — + *V. excelsa* POIR. (2n = 14). — ○ *V. excelsa* POIR. (2n = 56).

6—11 Paar beträgt; das Endblättchen ist ebenso gross wie die übrigen Blättchen. Die Blüten sind klein: höchstens 3 mm. lang. Von Individuen von den eingezeichneten Fundorten sind Wurzelspitzen fixiert worden. Die somatische Zahl für *V. officinalis* wurde von mir auf 14

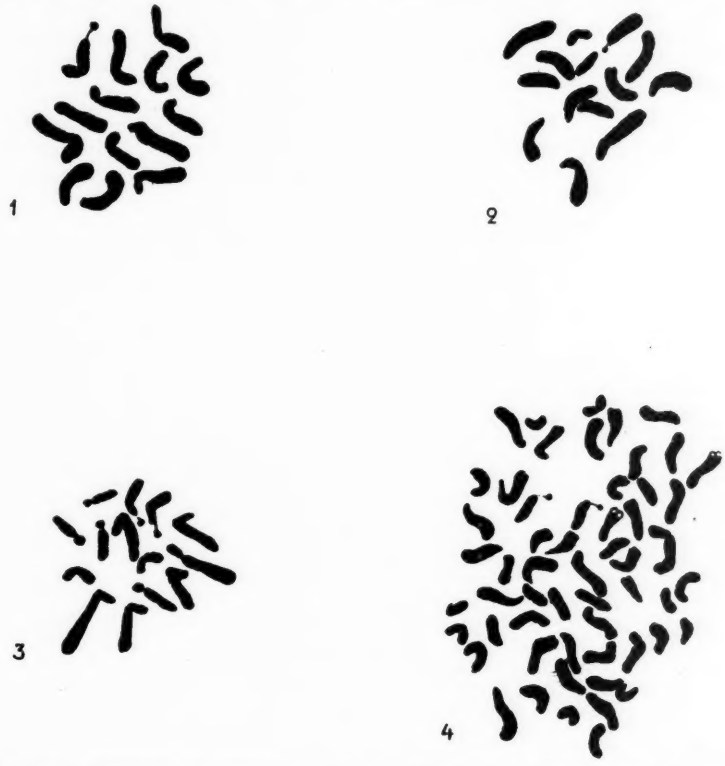


Fig. 1—4. Verschiedene somatische Platten. Fig. 1, *V. officinalis* aus Albrunna, Öland (2 = 14); Fig. 2, *V. baltica* aus Västervik, Sm. (2n = 14); Fig. 3, *V. balt.* × *off.* aus Gothenburg (2n = 14). × 4000; Fig. 4, *V. excelsa* aus Källstorp, Schonen (2n = 56). × 2800.

festgestellt. Wie aus Fig. 1 hervorgeht, kommt auch ein Satellitchromosom vor. Die abgebildete Platte stammt von einer Pflanze aus Albrunna auf Öland. Die übrigen untersuchten Exemplare von anderen Lokalen hatten auch mit Sicherheit ein Satellitchromosom. MEURMAN (1925 a, b) hat bei *V. officinalis* gefunden, dass n = 14 und die diploide Zahl = 28 ist (1931). Ob die von ihm untersuchten Typen Satelliten

hatten oder nicht, ist nicht angegeben; auch der Fundort wird nicht genannt. Wahrscheinlich handelt es sich aber um finnische Exemplare. *V. officinalis* hat also zwei Chromosomenrassen mit 14, bzw. 28 Chromosomen. Die 14-chromosomige Rasse scheint, nach bisher gemachten Untersuchungen, in Schweden und vielleicht auch in Norwegen verbreitet zu sein, während die 28-chromosomige in Finnland vorkommt. Dies unter der Voraussetzung, dass meine obige Vermutung richtig ist.

Eine *V. officinalis* nahestehende und wegen ihres systematischen Wertes diskutierte Art ist *V. baltica* PLEIJEL (= *V. officinalis* v. *simplicifolia* LEDEB.). Diese Art kommt jetzt nur mehr auf der Insel Lucernan bei Västervik (Schweden) und in Finnland (kürzlich dort

Fig. 5—6. Somatische Platten. Fig. 5, *V. excelsa* aus Svanö, Ångermanland (2n = 14). × 2800; Fig. 6, *V. salina* aus Jermansö, Ång. × 4000.

gefunden) vor, denn sie ist laut Angabe (PLEIJEL, 1907) an dem Ort in Livland, wo sie zuerst gefunden wurde, ausgestorben. *V. baltica* unterscheidet sich von *V. officinalis* hauptsächlich durch ganze Stengelblätter. Langjährige Anbauversuche zeigen, dass sie ein morphologisch konstanter Typus ist. Die diploide Zahl wurde von mir auf 14 bestimmt (Fig. 2). Ebenso wie *V. officinalis* hat auch *V. baltica* einen Satellit und dessen Chromosomenanordnung gleicht im übrigen sehr derjenigen von *V. officinalis*. — Die Kreuzung zwischen *V. officinalis* und *V. baltica*, die zum ersten Mal von PLEIJEL (1907) beschrieben wurde, ist in der Natur auf der oben erwähnten Insel Lucernan verwirklicht. Aber ausserdem kommen Hybriden in solchen Gärten vor, wo die beiden Elternarten nebeneinander angebaut wurden. Alle denkbaren Übergänge zwischen den Eltern kommen vor (siehe Photo, PLEIJEL, 1907). Der

vorherrschende Typus ist intermediär, d. h. mit einem grossen Endblättchen und 1—3 Paar Blättchen. — Eine intermediäre Form mit drei Paar Blättchen aus dem Botanischen Garten in Gothenburg habe ich untersucht und dabei gefunden, dass die diploide Zahl = 14 ist (Fig. 3). Zwei Satelliten sind deutlich sichtbar. Ein Teil der Chromosomen konnten als von *V. officinalis* herrührend identifiziert werden, andere wieder von *V. baltica*. Wahrscheinlich haben beide Eltern je zwei

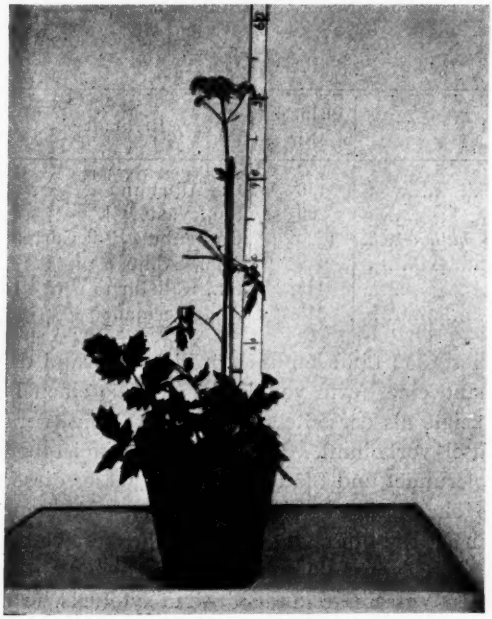


Fig. 7. *Valeriana excelsa* POIR. aus Svanö, Ång. (2n = 14); 50 cm. hoch.

Satellitchromosomen, aber das konnte noch nicht mit Sicherheit festgestellt werden. Die Hybriden sollten also einen Satellit von jeder der Elternart erhalten haben.

Die dritte von mir untersuchte Art ist *V. excelsa*. Im Gegensatz zu *V. officinalis* ist *V. excelsa* überwiegend eine Art des Binnenlandes. Sie ist über ganz Skandinavien und Finnland verbreitet. An der schwedischen Westküste ersetzt sie *V. officinalis*, ist aber dafür selten an der südschwedischen Ostküste zu finden. — *V. excelsa* unterscheidet sich von *V. officinalis* durch ihre verhältnismässig grossen Blüten; sie sind 5—6 mm. lang und das Endblättchen ist bedeutend kräftiger als die

übrigen Blättchen; von den letzteren gibt es in der Regel 5 Paar. Diese Art ist kräftiger als *V. officinalis*. Die nördlichen Exemplare haben im allgemeinen eine geringere Blattmasse als die südlichen. Die diploide Zahl für diese oben beschriebene Art ist 56 (Fig. 4). SENJANINOVA (1927) und MEURMAN (1931) haben früher dieselbe Zahl gefunden. Ich habe zwei Satelliten (Fig. 4) gefunden, die SENJANINOVA (1927) in ihrem Material nicht hat feststellen können. Die übrigen Chromosomen gleichen den von SENJANINOVA abgebildeten.

TABELLE 1.

Art	Pflanzen-N:r	Herkunft	2n	Sat.
V. officinalis	106	Öl., Albrunna	14	1 (2?)
V. baltica	200	Sm., Västervik	14	1 (2?)
V. officinalis × *baltica*	1200	Gothenburg, Bot. Garten	14	2
V. excelsa.....	418	Ång., Svanö	14	1 (2?)
V. excelsa.....	415: 2	Sk., Källstorp	56	2
V. salina	318	Ång., Jermansö	56	2

Im Sommer 1932 fand ich am nördlichen Strand der Insel Svanö in Ångermanland eine Form von *V. excelsa*, die einen bedeutend zarteren Wuchs zeigte als der 56-chromosomige Typus, der am südlichen Strand der Insel vorkommt. Das Endblättchen ist bei dieser Form breit bis oval gerundet und Blättchen gibt es ausser diesem gewöhnlich 3 Paar. Die Höhe beträgt 0,3—0,6 m. (Fig. 7). Die Stengel sind etwas anthocyangefärbt. Die Blüten haben ungefähr dieselbe Grösse wie beim 56-chromosomigen Typus. Sämtliche Pflanzen sind am offenen Strand in der Nähe des Wassers gestanden. Eine zytologische Untersuchung, die ich vornahm, hat ergeben, dass diese Form von *V. excelsa* in den somatischen Zellen nur 14 Chromosomen (Fig. 5) hatte. Nur ein Satellit konnte mit Sicherheit nachgewiesen werden (Fig. 5). — Auch in der Art *V. excelsa* gibt es also zwei Chromosomenrassen, deren Vertreter durch ihre morphologischen Eigenschaften voneinander unterschieden werden können. Die obige Beschreibung der beiden Rassen bildet noch eines der vielen Beispiele für positive Korrelation zwischen Chromosomenzahl und Gigas-Form. Die 14-chromosomige Rasse ist ein Strandökotypus, während die 56-chromosomige zu überwiegendem Teil ein Hainökotypus ist. Die beiden Rassen sind mit anderen Worten ökologisch getrennt (MÜNTZING, 1936). Ebenso verhält es sich mit den früher genannten *V. officinalis*. — Getrennt voneinander durch einen

grossen Waldbestand ist die Hybridisierung zwischen den beiden Arten auf dem nördlichen und südlichen Teil der Insel ausgeschlossen.

Wie früher erwähnt wurde, scheidet PLEIJEL (1925) eine neue Art aus: *V. salina*. Diese Art fasst er als eine hybridogene Form zwischen *V. officinalis* und *excelsa* auf. *V. salina* ist mit ihrem kräftigen Endblättchen und ihren 6—11 Paar Blättchen intermediär. Die Blätter sind dick, ziemlich hart und glänzend; die äussere Erscheinung der Art deutet auf eine Anpassung an den Meeresstrand hin. — Vom nördlichen Teil des Regierungsbezirkes Kalmar und der Schären Södermanlands, wo sie ganz vorherrscht, breitet sich diese Art rund um den Bottnischen Meerbusen, teilweise auf dem nördlichen Strand des Finnischen Meerbusens und auf Åland aus. Nach den Untersuchungen von ASPLUND (1920) und MEURMAN (1925 b, 1931) hat *V. salina* n = 28 und 2n = 56. Sämtliche von mir untersuchten Exemplare von drei Fundorten hatten auch die diploide Zahl 56 (Fig. 6), wobei Chromosomen Satelliten trugen. Die hohe Chromosomenzahl verleiht aber der Pflanze keinen eigentlichen Gigas-Charakter, sondern dieser kommt mehr in der Dicke der Blätter zum Ausdruck. Irgendwelche Chromosomenrassen wurden also nicht gefunden, sondern *V. salina* scheint als eine einheitliche Art in den schwedischen und finnischen Schären vorzuherrschen. Dass *V. salina* ein Meeresstrand- oder ein Klippenökotypus ist, darüber besteht kein Zweifel, aber ob sie eine Hybride zwischen *V. officinalis* und *V. excelsa* ist, kann vielleicht eine weitere Untersuchung zeigen.

Svalöf, im Dezember 1936.

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GENSTUDIEN AN PISUM SATIVUM

III. ÜBER DEN EFFEKT DES GENPAARES Gl—gl

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(With a summary in English)

EINLEITUNG.

WAS bisher über das Genpaar *Gl—gl* bekannt ist, verdanken wir H. und O. TEDIN (1928). Diese Verfasser fanden eine Linie, 0881, herstammend aus der deutschen Sorte »Frühe gelbe Thüringer Erbse» mit rosafarbigen Blüten, die sich dadurch auszeichnete, dass der die Radikula bedeckende Teil der Samenschale eine orangegelbe Farbe zeigte. Ein paar solche Samen sind von den genannten Verfassern l. c. auf der farbigen Tafel unter Nr. 38 wiedergegeben.

In bezug auf den Einfluss des Gens *Gl* auf die Färbung der Samenschale sind in der zitierten Arbeit von H. und O. TEDIN folgende Angaben zu finden. Das Auftreten der orangegelben Testafarbe an der Radikulastelle wird auf die Wirkung des Gens *Gl* zurückgeführt. Zur Feststellung der Vererbungsverhältnisse dieses Samencharakters haben genannte Verfasser eine Kreuzung ausgeführt zwischen oben erwähnter Linie 0881 und Linie 0652, die aus der dänischen Sorte Glaenö stammt. Für Linie 0881 wird die Genenformel *A Ar b D pl m f Gl Oh Z mp Fl i* angegeben, für 0652 *A Ar B D pl M f gl Oh z Mp Fl I*. Nach diesen Formeln zu schliessen sollte die genannte Eigenschaft der Linie 0881 also auf die Wirkung der homozygot dominanten Form des Gens *Gl* zurückzuführen sein.

Über die Kreuzungsergebnisse führen H. und O. TEDIN an, dass sie bei weitem nicht klarlegend sind. F_1 zeigte marmorierte Samen und an diesen konnte die Eigenschaft orangegelbe Radikula nicht festgestellt werden. In F_2 gab es 141 Individuen mit nicht marmorierten Samen, von denen 38 rosa Blütenfarbe (*A Ar b*) hatten, die alle Samen mit dem genannten Charakter zeigten. Unter den purpurblütigen gab es nur wenige mit dieser Eigenschaft. Ferner soll die orangegelbe Testafarbe über der Radikula in ihrer Ausbildung sehr stark variieren und bei grüner Keimblattfarbe besser zutage treten als bei gelber.

In der dritten Generation wurden insgesamt 129 Familien untersucht, die aber auch nicht zu klareren Resultaten geführt haben. Die Verfasser sagen hier: »The analysis of the segregation within the families was impossible. It was not even possible to decide whether the yellow radicula was dominant or not. In some families there was a rather distinct segregation in yellow : not yellow, in others the segregation was completely continuous and sometimes with most plants clearly yellow, sometimes with most plants dubious or not yellow. It is evident, that even if one main factor exists, there is a considerable number of disturbing modifiers». Die Verfasser bezeichnen das Gen *Gl* daher auch als einen »New hypothetical factor», und setzen das Symbol (*Gl*) in Klammern.

Eine Erscheinung hat die Analyse der F_3 indessen sehr wahrscheinlich gemacht, nämlich dass der in Frage stehende Charakter mit dem Gen *B* ziemlich stark gekoppelt ist.

Schliesslich heben H. und O. TEDIN (l. c.) die Möglichkeit hervor, dass für das Auftreten des genannten Charakters mehrere Gene verantwortlich sein können. Als besonders hierfür sprechend führen sie an, dass in Kreuzungen, wo keiner der Eltern die Eigenschaft orangegelbe Testa über der Radikula zeigte, in F_3 und F_4 Pflanzen mit dieser aufgetreten sind. Diese sind leider nicht weiter untersucht worden.

EIGENE KREUZUNGSRESULTATE.

Als Elter mit der Eigenschaft orangegelbe Testa über der Radikula ist mir die gleiche Linie 0881 zur Verfügung gestanden, die die oben genannten Verfasser verwendet haben. Eine Samenprobe dieser ist mir vom verstorbenen Dr. H. TEDIN in liebenswürdiger Weise übergeben worden. Sie wurde in meiner Liniensammlung als L 22 registriert. Zwecks Klarlegung und weiteren Studiums der Vererbung des in Frage stehenden Charakters wurde L 22 mit drei anderen gekreuzt. Der Elter einer dieser drei Kreuzungen, von Kreuzung Nr. 224, die hier ausschliesslich besprochen werden soll, war L 206, aus einer niedrigen Brechmarkerbse. Für die genotypische Konstitution dieser Linie kann folgende Formel angegeben werden: *A Ar B D pl m F Oh Z Fl r I le v n.* In bezug auf die beiden Genpaare *Gl—gl* und *Mp—mp* war die Konstitution von L 206 unbekannt. Zu erwähnen ist in diesem Zusammenhang, dass in Kreuzungen zwischen L 206 und Linien mit gefärbter Testa (*A*) keine Samen mit orangegelber Radikula aufgetreten sind, woraus man — unter der Voraussetzung, dass dieser Charakter durch

ein dominantes Gen bedingt wird — schliessen darf, dass L 206 die Konstitution *gl* zukommt. Die Genenformel, die oben in der Einleitung für L 22 (0881) angegeben worden ist, ist hier noch zu ergänzen durch *R Le V N*.

Auf Grund des vorstehend über die genotypische Konstitution von L 22 und L 206 angeführten ist in Kreuzung Nr. 224 eine Spaltung in wenigstens folgenden Genpaaren zu erwarten: *A—a, F—f, Gl—gl, R—r, I—i, B—b, Le—le, V—v* und *N—n*, also eine Spaltung in neun Genpaaren.

Die im Jahre 1935 gebaute erste Generation bestand aus 28 Pflanzen, die insgesamt 1464 gut entwickelte Samen gegeben haben. An F_1 konnten folgende Eigenschaften festgestellt werden: Hoher Wuchs (*Le*), Purpurfarbige Blüten (*A Ar B*), panachierte Stipeln und Blättchen (*Fl*), einfacher Anthozyanring um die Stipelbasis (*D*), Hülsen mit starker Membran und der gewöhnlichen Wandstärke und Form (*P V N*), Samenschale in ihrer Gänze gefärbt, nicht rot, nicht marmoriert, violett punktiert, mit gelblichbraunem, normal entwickeltem Hilum, aber ohne jede Andeutung von orangegelber Farbe an der Stelle der Radikula (*A Ar pl m F Gl Oh Z*); die Samenform spaltet in *R—r* und *I—i*, *A RR*- und *A Rr*-Samen sind indent (*Z Rr Ii*).

Sämtliche an F_1 bzw. F_2-Samen feststellbaren Charaktere stimmen also, mit Ausnahme hinsichtlich des Genpaares *Gl—gl*, damit überein, was auf Grund der oben für die Eltern angegebenen genotypischen Formeln zu erwarten gewesen ist. Da die F_1-Samenschale in der vorliegenden Kreuzung nicht marmoriert ist, sollte die Wirkung von *Gl* leicht zu beobachten sein. Kein Same zeigte indessen den in Frage stehenden Charakter. Schon diese Feststellung allein scheint mir zu genügen, um den von H. und O. TEDIN (l. c.) als orangegelbe Radikula bezeichneten Charakter als zweifellos rezessiv aufzufassen. L 206 sollte demnach die Konstitution *Gl*, L 22 (0881) *gl* zukommen.

Vor Besprechung der in F_2 erhaltenen Spaltungsergebnisse bringe ich eine kurze Charakteristik der Eigenschaft orangegelbe Radikula, wie sie in L 22 und an Samen der F_2 von Kreuzung 224 zutage tritt. Die Bezeichnung Orangegelb ist keine adequate, da diese eine reine Regenbogenfarbe ist, während die *gl gl* entsprechende Farbe als Ockergelb zu bezeichnen ist. Sie entspricht in R. RIDGWAY, Color Standards and Color Nomenclature (1912) in der Mehrzahl der Fälle genau Yellow Ocher, XV, 17′. Zuweilen ist sie etwas heller, nur selten etwas dunkler.

Die Ausbreitung der durch *gl gl* bedingten lokalen Testafärbung geht deutlich aus H. und O. TEDINS Fig. 38 auf Tafel I hervor. Die

Färbung deckt die Stelle, unter der die Radikula gelegen ist und setzt beiderseits des Hilums in der Form einer schmalen Einfassung dieses fort. Mitunter ist die gefärbte Stelle etwas breiter als die Radikula. Gleichzeitig mit dem Auftreten dieser Färbung bekommt die Nabelwarze, die Caruncula, eine dunklere, braune Färbung. Die Caruncula liegt bei *Pisum*, in gleicher Weise wie bei *Phaseolus*, auf der der Mikropyle gegenüber gelegenen Seite des Hilums, ist vom Rand des letzteren gewöhnlich etwa 3 Millimeter entfernt, aber mit diesem durch einen Gefässstrang verbunden. H. und O. TEDIN benutzten für diese Nabelwarze bei *Pisum* die Bezeichnung *Spermotylium*.

Mit Hinsicht auf die Variation in der Ausbildung der in Frage stehenden lokalen Testafärbung haben H. und O. TEDIN, wie schon in der Einleitung erwähnt, alle Übergänge zwischen Abwesenheit und deutlicher Ausbildung derselben gefunden, was in ihrer Kreuzung die Klassifikation derart erschwert hat, dass sie nicht einmal entscheiden konnten, ob der Charakter eine dominante oder rezessive Eigenschaft ist. Eine solche Variation könnte sowohl durch Milieuverhältnisse wie durch Einfluss der sonstigen, verschiedenen genotypischen Konstitution bedingt sein. Meine Resultate haben diesbezüglich u. a. folgendes zutage gebracht. Schlecht ausgebildet ist diese lokale Färbung der Testa gewöhnlich dann, wenn die Samen nicht gut ausgereift sind. Samen mit gelber Kotyledonenfarbe, dem Gen *I* entsprechend, bekommen, wenn sie schlecht ausgereift sind, bekanntlich eine grünlich gelbe bis gelblich grüne Farbe, und es kann vorkommen, dass man bei solchen Samen im Zweifel ist, ob es sich um gelbe oder grüne Kotyledonenfarbe handelt. An Pflanzen in Kreuzung 224, wo zum Teil solche Samen vorgekommen sind, wurde gleichzeitig beobachtet, dass die durch *gl gl* verursachte lokale Testafärbung schlecht ausgebildet oder gar kaum zu erkennen war.

Vielleicht sind die Klassifikationsschwierigkeiten, mit denen H. und O. TEDIN zu kämpfen hatten, grossenteils auf eine solche durch Milieuverhältnisse bedingte Variation zurückzuführen. Bei der in Rede stehenden Klassifikation in F_2 meiner Kreuzung Nr. 224 haben sich praktisch genommen keine Schwierigkeiten ergeben. Sowohl bei gelber wie bei grüner Kotyledonenfarbe war gewöhnlich unmittelbar zu erkennen ob die Ockergelbe Farbe über der Radikula vorhanden war oder nicht. Deshalb ist natürlich nicht ausgeschlossen, dass im Falle von H. und O. TEDIN auch genische Verhältnisse mitgewirkt haben. Solche Fälle scheinen übrigens nicht geradezu selten zu sein. So ist die Klassifikation im Genpaar *F—f* (punktierte—nicht punktierte Testa)

in vorliegender Kreuzung sehr schwierig und daher unsicher gewesen. Gleiches gilt meistens für die Klassifikation gerade — gekrümmte Hülse bei *Pisum* (Genpaar *Cp—cp*). In einer meiner Kreuzungen hat diese Klassifikation keine Schwierigkeiten verursacht, in den meisten anderen war sie aber schwierig. Meiner Ansicht nach ist die Ursache für diese Erscheinung gewöhnlich darin zu suchen, dass die im in Rede stehenden Gen Heterozygoten durch die übrige genische Konstitution so beeinflusst werden, dass die Dominanz keine vollständige wird: es treten intermediäre Typen mit Übergängen nach beiden Seiten hin auf.

Hier ist noch zu erwähnen, dass die Konstitution *gl gl* ausser durch die lokale Färbung der Testa in meinem Material sich auch in einer gewissen, wenngleich schwachen Beeinflussung der übrigen Testafarbe äussert. Der Farbenton erhält sowohl bei Samen mit gelber wie mit grüner Kotyledonenfarbe eine schwache Beimengung von Gelblichgrau. In Kreuzung Nr. 224 konnten *gl gl*-Samen daher gewöhnlich unmittelbar als solche erkannt werden, noch ehe man der lokalen Ockergelben Testafarbe seine Aufmerksamkeit zugewendet hatte.

Für die in der zweiten Generation gefundenen Spaltungen ist das Originalmaterial in Tab. 1 für die fünf Genpaare *V—v*, *N—n*, *Le—le*, *A—a* und *Gl—gl* mitgeteilt. Die Spaltung im Genpaar *F—f* ist wegen der bereits erwähnten Klassifikationsschwierigkeiten nicht berücksichtigt und soll erst nach Untersuchung einer weiteren F_2 und Überprüfung in F_3 veröffentlicht werden. Die Spaltung im Genpaar *B—b* (purpur- bzw. rosafarbige Blüten) kann leider nicht angegeben werden, da die betreffende Beurteilung infolge eines Missverständnisses unterblieben ist. Die Spaltung in den beiden Genpaaren *R—r* und *I—i* ist in der Tabelle 1 — um sie nicht unnötig gross zu machen — nicht aufgenommen, wird aber in allen ihren bifaktoriellen Kombinationen weiter unten angegeben.

Insgesamt wurden in F_2 500 Samen gesät, die 443, das sind 88,7 %, in jeder Hinsicht beurteilbare Pflanzen entwickelt haben, was noch als normal zu betrachten sein dürfte. Irgendwelche partielle oder Semisterilität hat in dieser Kreuzung nicht beobachtet werden können.

Für die einzelnen Genpaare wurden folgende Spaltungsverhältnisse erhalten.

Gefunden:	353 *V* :	90 *v*	Gefunden:	269 *Gl* :	69 *gl*
Erwartet:	332,25 » :	110,75 »	Erwartet:	253,50 » :	84,50 »
D/m für 3 : 1 =	2,28		D/m für 3 : 1 =	1,95	

TABELLE 1. *Die Spaltung in den fünf Genpaaren V—v, N—n, Le—le, A—a und Gl—gl in der zweiten Generation der Kreuzung Nr. 224, L. 22 aus Thüringer Erbse × L. 206, Brechmarkerbse.*

Familie-Nr.	V													v												Summe
	N							n						N							n						
	Le			le				Le			le			Le			le				Le			le			
	A		a	A		a		A		a	A		a	A		a	A		a		A		a	A		a	
	Gl	gl		Gl	gl			Gl	gl		Gl	gl		Gl	gl		Gl	gl			Gl	gl		Gl	gl		
8439	28	12	19	1	1	—	61	18	3	3	—	—	—	24	3	—	2	5	1	4	15	—	—	—	4	—	—	4	104
8440	13	3	1	1	—	—	18	4	2	1	—	—	—	7	—	—	—	3	—	1	4	—	—	—	2	—	1	3	32
8441	2	—	4	—	—	—	6	1	—	—	—	—	1	2	—	—	—	3	1	1	5	—	—	—	—	—	1	1	14
8442	1	1	—	—	—	—	2	2	—	1	—	—	—	3	—	—	—	1	—	1	2	1	—	—	—	—	—	1	8
8443	21	7	9	—	—	—	37	10	2	5	—	—	—	17	1	—	—	9	—	3	13	—	—	—	4	1	—	5	72
8444	6	1	2	—	—	—	9	1	—	2	—	—	—	3	1	—	—	—	—	—	1	—	—	—	1	—	—	1	14
8445	18	10	5	1	—	—	34	12	5	7	—	—	1	25	1	—	—	7	2	2	12	1	—	—	—	—	—	1	72
8446	5	1	2	—	—	—	8	1	—	1	—	—	—	2	—	—	—	—	1	—	1	—	—	—	—	—	—	—	11
8447	14	3	4	2	—	—	23	2	—	—	—	—	—	2	—	—	—	1	—	—	1	—	—	—	—	—	—	—	26
8448	2	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	3
8449	23	5	14	3	1	—	46	7	1	1	2	—	—	11	1	1	—	5	1	1	9	—	—	—	2	1	1	4	70
8450	6	1	1	—	—	—	8	1	1	—	1	—	—	3	—	—	—	4	1	—	5	—	—	—	—	—	1	1	17
Summen:	139	44	61	8	2	—	254	59	14	21	3	—	2	99	7	1	3	38	6	14	69	2	—	—	13	2	4	21	443

Gefunden:	323 *N* : 120 *n*	Gefunden:	359 *R* : 84 *r*
D/m für 3 : 1 =	1,01	D/m für 3 : 1 =	2,94
Gefunden:	351 *Le* : 92 *le*	Gefunden:	328 *I* : 115 *i*
D/m für 3 : 1 =	2,06	D/m für 3 : 1 =	0,47
Gefunden:	338 *A* : 105 *a*		
D/m für 3 : 1 =	0,63		

In den drei Genpaaren *V*—*v*, *Le*—*le* und *R*—*r* weicht die Spaltung stärker von den erwarteten Zahlen ab, indem die Rezessiven hier ein gewisses Defizit aufweisen. Bei Zuckererbsen, den *vv*-Typen, ist dies eine ziemlich häufige Erscheinung, sie sind gewöhnlich weniger vital als Kneifelerbsen. Und bei den *le le*-Typen, den niedrigen Pflanzen, dürfte hier dem Umstand eine gewisse Bedeutung zukommen, dass diese in F_2 stets zwischen hohen Pflanzen (*Le*) zu stehen kommen, und dadurch in ihrer Entwicklung nicht wenig gehindert werden. Schliesslich ist für Samen vom Markerbsentypus (*rr*) seit langem bekannt, dass sie ungünstigen Witterungseinflüssen gegenüber weitaus empfindlicher sind als *R*-Samen. Wenn daher auch bei der Analyse der F_2-Samen eine fehlerfreie monohybride Spaltung in *R*—*r* gefunden wird, so werden dann doch meistens weniger entwickelte *rr*-Pflanzen angetroffen, ein Teil der *rr*-Samen ist eben zugrunde gegangen.

Auch die *gl gl*-Pflanzen zeigen ein geringeres Defizit. Über dessen eventuelle Ursache kann indessen bisher nichts ausgesagt werden. Das Genpaar *Gl*—*gl* zeigt hier im übrigen eine klare monohybride Spaltung, wodurch wohl einwandfrei festgestellt erscheint, dass die Eigenschaft Ockergelbe Testa über der Radikula durch Rezessivität des Gens *Gl*, *gl gl*, bedingt wird. Ferner konnte bestätigt werden, dass *gl gl* bei durch *a a* bedingter farbloser Testa keinen Effekt auf die Färbung dieser hat.

Im Folgenden werden die in Kreuzung 224 bisher festgestellten dihybriden Spaltungsverhältnisse kurz zahlenmässig mitgeteilt. Wenn die Spaltungszahlen infolge Ausfall von Rezessiven stärkere, statistisch unwahrscheinliche Abweichungen zeigen, werden auch die auf Grund der monohybriden Spaltungsverhältnisse zu erwartenden Zahlen angegeben.

Gefunden: 254	*V N* : 99	*V n* : 69	*v N* : 21	*v n*
Erwartet: 249,19	» » : 83,06	» » : 83,06	» » : 27,69	» »
D/m für 9 : 3 : 3 : 1 =	+ 0,46	+ 1,94	— 1,71	— 1,30

Gefunden: 338 *V Le* : 15 *V le* : 13 *v Le* : 77 *v le*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 8,50 — 8,29 — 8,53 + 9,69

Gefunden: 269 *V A* : 84 *V a* : 69 *v A* : 21 *v a*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 1,90 + 0,11 — 1,71 — 1,30

Gefunden: 209 *V Gl* : 60 *V gl* : 60 *v Gl* : 9 *v gl*
Erwartet: 190,12 » » : 63,37 » » : 63,37 » » : 21,12 » »
D/m für
9 : 3 : 3 : 1 = + 2,07 — 0,47 — 0,47 — 2,73

Erwartet auf Grund der monohybriden Spaltungszahlen:
214,3 : 55,0 : 54,7 : 14,0
D/m = — 0,51 + 0,61 + 0,64 — 1,40

Gefunden: 288 *V R* : 65 *V r* : 71 *v R* : 19 *v r*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 3,72 — 2,20 — 1,47 — 1,70

Erwartet auf Grund der monohybriden Spaltungszahlen:
286,1 : 66,9 : 72,9 : 17,1
D/m = + 0,19 — 0,23 — 0,23 + 0,37

Gefunden: 260 *V I* : 93 *V i* : 68 *v I* : 22 *v i*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 1,04 + 1,21 — 1,83 — 1,10

Gefunden: 255 *N Le* : 68 *N le* : 96 *n Le* : 24 *n le*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 0,56 — 1,83 + 1,58 — 0,71

Gefunden: 245 *N A* : 78 *N a* : 93 *n A* : 27 *n a*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = — 0,40 — 0,62 + 1,21 — 0,12

Gefunden: 192 *N Gl* : 53 *N gl* : 77 *n Gl* : 16 *n gl*
Erwartet: 190,12 » » : 63,37 » » : 63,37 » » : 21,12 » »
D/m für
9 : 3 : 3 : 1 = — 0,21 — 1,45 + 1,90 — 1,15

Gefunden: 264 *N R* : 59 *N r* : 95 *n R* : 25 *n r*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 1,42 — 2,93 + 1,46 — 0,52

Gefunden: 239 *N I* : 84 *N i* : 89 *n I* : 31 *n i*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = — 0,97 + 0,11 + 0,72 + 0,62

Gefunden: 266 *Le A* : 85 *Le a* : 72 *le A* : 20 *le a*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 1,66 + 0,24 — 1,34 — 1,50

Gefunden: 207 *Le Gl* : 59 *Le gl* : 62 *le Gl* : 10 *le gl*
Erwartet: 190,12 » » : 63,37 » » : 63,37 » » : 21,12 » »
D/m für
9 : 3 : 3 : 1 = + 1,85 — 0,61 — 0,19 — 2,50

Gefunden: 284 *Le R* : 67 *Le r* : 75 *le R* : 17 *le r*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 3,34 — 1,95 — 0,98 — 2,09

Erwartet auf Grund der monohybriden Spaltungszahlen:
284,5 : 66,5 : 74,5 : 17,5
D/m = — 0,05 + 0,06 — 0,06 — 0,10

Gefunden: 263 *Le I* : 90 *Le i* : 65 *le I* : 25 *le i*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 1,32 + 0,84 — 2,20 — 0,52

Gefunden: 269 *A Gl* : 69 *A gl* : 105 $a\,\frac{Gl}{gl}$
Erwartet: 249,19 » » : 83,06 » » : 110,75 » »
D/m für
9 : 3 : 4 = + 1,90 — 1,71 — 0,63

Gefunden: 272 *A R* : 66 *A r* : 87 *a R* : 18 *a r*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = + 2,18 — 2,08 + 0,48 — 1,90

Gefunden: 245 *A I* : 93 *A i* : 83 *a I* : 22 *a i*
Erwartet: 249,19 » » : 83,06 » » : 83,06 » » : 27,69 » »
D/m für
9 : 3 : 3 : 1 = — 0,40 + 1,21 — 0,01 — 1,10

Gefunden: 213 *Gl R* : 56 *Gl r* : 59 *gl R* : 10 *gl r*
Erwartet: 190,12 » » : 63,37 » » : 63,37 » » : 21,12 » »
D/m für
9 : 3 : 3 : 1 = + 2,51 — 1,03 — 0,61 — 2,50

Gefunden: 193 *Gl I* : 76 *Gl i* : 52 *gl I* : 17 *gl i*
Erwartet: 190,12 » » : 63,37 » » : 63,37 » » : 21,12 » »
D/m für
9 : 3 : 3 : 1 = + 0,32 + 1,76 — 1,58 — 0,93

Ein Durchgehen der vorstehend mitgeteilten Zweigenenspaltungen in Kreuzung Nr. 224 ergibt, dass unter diesen nur ein sicherer Fall von Koppelung zu konstatieren ist, nämlich die bereits seit langem bekannte Koppelung zwischen den Genen *Le* und *V*. Eine Berechnung des Koppelungsgrades auf Grund der erhaltenen Spaltungszahlen ergibt 6,53 % Crossing-over. Die bifaktoriellen Spaltungszahlen zeigen ein für Koppelung sehr schönes, gut balanziertes Bild, indem D/m für die beiden mittleren Glieder — 8,29 bzw. — 8,53 beträgt. Auch eine Berechnung des zu erwartenden Spaltungsverhältnisses auf Grund der beiden monohybriden Verhältnisse ändert nichts wesentliches in diesem Bilde. Dann resultieren nämlich folgende Zahlen.

Gefunden: 338 *V Le* : 15 *V le* : 13 *v Le* : 77 *v le*
Erwartet: 279,7 » » : 73,3 » » : 71,3 » » : 18,7 » »
D/m für
9 : 3 : 3 : 1 = + 5,58 — 7,10 — 7,10 + 11,4

Hier zeigen die beiden mittleren Glieder im Verhältnis, den Umkombinationen entsprechend, ein noch schöneres, man möchte sagen unnatürlich gleichmässiges Defizit, indem D/m für beide Glieder denselben Wert von — 7,10 erreicht. Das Defizit an *le le*- bzw. *v v*-Pflanzen, wie es oben aus den monohybriden Spaltungsverhältnissen hervorgeht, scheint hierauf also keinen nennenswerten Einfluss ausgeübt zu haben.

Hier liegt eine für die beiden Gene *Le* und *V* noch nicht konstatierte Stärke von Koppelung vor. Die bisher hierfür veröffentlichten Crossing-over-Werte (RASMUSSON, 1927; WELLENSIEK, 1929) liegen zwischen etwa 11 und 22 %. Ohne hier näher auf diese Erscheinung einzugehen, die

ich binnen kurzem zum Gegenstand einer Studie zu machen gedenke, sei nur erwähnt, dass ich als Erklärung hierfür einen Einfluss der übrigen genischen Konstitution des Materials annehme.

Das speziell studierte Gen *Gl* hat mit keinem der hier analysierten Gene eine sichere Koppelung gezeigt, was damit im Einklang steht, dass es, laut H. und O. TEDIN (l. c.), mit *B* stark gekoppelt ist; und im *B*-Chromosom ist keines der übrigen hier untersuchten Gene gelegen, wenigstens nicht wenn es sich um den gewöhnlichen, den anscheinend häufigsten *B*-Chromosomentypus handelt.

Alle übrigen hier studierten Gene zeigen, abgesehen von den durch ein in gewissen Fällen vorkommendes Defizit an Doppeltrezessiven hervorgerufenen Abweichungen, normale bifaktorielle Spaltungsverhältnisse und damit voneinander unabhängige Vererbung.

DAS AUFTRETEN VON gl gl-TYPEN ALS REZESSIV-MUTANTEN.

In einer meiner Kreuzungen, Nr. 59, über die schon im Zusammenhang mit der Besprechung des Genpaares *Uni—uni* früher zweimal berichtet worden ist (LAMPRECHT, 1933 a und b), ist in F_4 und F_5 je einmal spontan eine Pflanze aufgetreten, deren Samen alle deutlich den Charakter Ockergelbe Testa über der Radikula gezeigt haben. Kreuzung 59 ist ausgeführt zwischen L 21, die identisch ist mit H. TEDINS 01001, und L 187, die im Gen *Uni* heterozygot ist. In dieser Kreuzung kam es zur Aufspaltung in folgenden 7 Genpaaren: *A—a*, *Ar—ar*, *B—b*, *M—m*, *Pl—pl*, *Le—le* und *Uni—uni*. Das Gen *uni* bedingt in seiner homozygot rezessiven Form bekanntlich die Reduktion des geteilten *Pisum*-Blattes auf ein einfaches oder ein 2—3-teiliges, wie wir es bei *Trifolium* finden. Näheres über diese Blattypen siehe LAMPRECHT, 1933 b, S. 280—282. Ausserdem haben *uni uni*-Pflanzen sämtliche Blütenelemente zu schmalen grünen Blättchen von mehr oder weniger ausgeprägtem pistilloidem Charakter umgebildet.

In F_4 von Kreuzung 59 hat nun im Jahre 1935 eine *gl gl*-Pflanze ausgespalten. Die Familie, in der dies eingetroffen ist, spaltete in den Genpaaren *M—m* und *Uni—uni* und war in folgenden Genen homozygot: *A, ar, b, Pl, Le*. Die in Frage stehende *gl gl*-Pflanze war nun auch in *m* homozygot. Sie gab insgesamt 58 Samen, von denen im Jahre 1936 30 ausgesät wurden. Diese haben in F_5 zusammen 23 voll entwickelte Pflanzen gegeben. Die in Rede stehende F_5-Familie von Kreuzung 59 (Nr. 8552/36) war also in 6 der 7 in F_2 spaltenden, oben angeführten

Genpaare homozygot und spaltete nur mehr in *Uni—uni*. In diesem Genpaar hat folgende Spaltung registriert werden können:

Gefunden:	17	*Uni* : 6	*uni*
Erwartet:	17,25	» : 5,75	»
D/m für 3 : 1 =	0,12		

Die durch *gl gl* bedingte Färbung der Samenschale kann natürlich an *uni uni*-Pflanzen nicht festgestellt werden, da diese infolge der erwähnten Umbildung der Blütenelemente steril sind. Ausser *gl gl* zeigten demnach alle diese Pflanzen die Konstitution *A ar b m Pl Le*, und hatten also durchweg Ockergelbe Testa über der Radikula.

Im vorliegenden Fall dürfte demnach wohl kaum ein Zweifel darüber bestehen, dass es in dieser Kreuzung zu einer Mutation im Genpaar *Gl—gl*, von Dominanz zu Rezessivität gekommen ist. Die Mutation sollte hiernach schon in F_2 stattgefunden haben, sodass auf dieser ein F_3-Same mit der Konstitution *Gl gl* erhalten worden ist, der dann auf der aus demselben entwickelten F_3-Pflanze zur Entstehung einer Anzahl von Samen mit der Konstitution *gl gl* geführt hat. Diese sind natürlich noch nicht als solche erkennbar, da die Samenschale noch der Elterngeneration, also F_3 mit der Formel *Gl gl* angehört. In F_4 ist dann eine Pflanze mit *gl gl* entstanden, deren Samen durchweg den in Frage stehenden Charakter gezeigt haben. Im Jahre 1936 ist in derselben Kreuzung eine solche Pflanze in F_5 gefunden worden (Nr. 8542—4/36), bei der also in Übereinstimmung mit obigem die Mutation erst in F_3 stattgefunden haben sollte. Auch in diesem Fall zeigte die in Frage stehende Pflanze alle Eigenschaften, durch die die Familie im übrigen charakterisiert wird.

Eine Verschiebung im Auftreten dieses Charakters um eine Generation wäre denkbar, wenn diese Mutation somatisch oder gleichzeitig in einer Samenanlage und in Pollenkörnern derselben Blüte auftreten würde, was aber wohl weniger wahrscheinlich sein dürfte.

Mit Hinblick auf die vorstehend mitgeteilten Beobachtungen über Mutation von *Gl* zu *gl* in meiner Kreuzung Nr. 59 hege ich keine Bedenken auch die von H. und O. TEDIN gemachte Beobachtung des Auftretens von Pflanzen mit dem Charakter Ockergelbe Testa über der Radikula in F_3 und F_4 gewisser Kreuzungen als solche aufzufassen. H. und O. TEDIN wollten hierfür anstatt dessen das Zusammenwirken mehrerer Gene für das Auftreten dieses Charakters annehmen.

Eine solche Annahme ist natürlich nicht ohne weiteres zurückzuweisen, aber solchenfalls besteht auch die Möglichkeit ihre ev. Richtig-

keit in einfacher Weise zu prüfen. Denn treten einmal in F_3 oder F_4 einzelne der in Frage stehenden Typen auf, so müssen wir in den folgenden Generationen — bei Richtigkeit der Annahme einer Mehrgenenspaltung als Ursache — unbedingt auf Familien bzw. Serien solcher stossen, in denen einfache Spaltungen im in Rede stehenden Charakter auftreten (15 : 1, 3 : 1). In meiner Kreuzung Nr. 59, die allerdings erst bis in F_5, aber doch insgesamt an etwa 5500 Individuen studiert worden ist, haben hierfür bisher keine Anhaltspunkte gefunden werden können. Bei Zugrundelegung einer Mehrgenenspaltung als Erklärung des Auftretens der Ockergelben Testa über der Radikula sollte dieser Charakter im vorliegenden Falle durch wenigstens 5-fache Rezessivität bedingt werden. Meines Erachtens handelt es sich zweifellos um eine Rezessivmutation. F_6 wird hierauf Antwort geben. Ferner lässt sich dies leicht durch Kreuzen der beiden Eltern von Kreuzung 59 mit L 22 (*gl gl*) beweisen, denn wenigstens eine der Elternlinien müsste solchenfalls in mehreren der Genpaare dominant sein und eine damit im Einklang stehende Spaltung geben.

ÜBER EINEN ZUSAMMENHANG ZWISCHEN DEM GENPAAR Gl—gl UND DEM SAMENERTRAG.

Bei der Beurteilung der Samen von Kreuzungspflanzen charakterisiere ich den Samenertrag stets durch eine Zahl: 1 = 1—5 Samen, 2 = 6—10, 3 = 11—15, 4 = 16—20, 5 = 21—30, 6 = 31—40 Samen u. s. w. Für über 50 Samen werden gewöhnlich Klassen von 20 Samen benutzt. Bei dieser Klassifikation des Samenertrages in vorliegender Kreuzung, Nr. 224, ist es mir sehr bald aufgefallen, dass sich unter den *gl gl*-Pflanzen zweifellos mehr mit höherem Ertrag befanden als unter den *Gl*-Pflanzen (sämtliche natürlich mit *A*). Dies veranlasste eine genauere Analyse, wobei auch sämtliche Pflanzen mit mehr als 50 Samen in 10 Samen-Klassen eingeteilt worden sind.

Die hierbei erhaltenen Resultate sind in Tabelle 2 mitgeteilt, und zwar verteilt auf drei Gruppen, *A Gl*-Individuen, *A gl gl*-Individuen und *a*-Individuen. In Fig. 1 sind die beiden ersten Gruppen, nach Umrechnung auf gleiche Individuenanzahl, graphisch dargestellt.

Schon ein Blick auf die Tabelle 2 dürfte zeigen, dass der Samenertrag der *A Gl*-Individuen durchschnittlich deutlich niedriger sein muss als der der *A gl gl*-Individuen. Für die drei Gruppen werden folgende Mittelwerte und dazu gehörige mittlere Fehler erhalten. Bei der Berechnung wurden die Zahlen 5, 15, 25, 35, 45, 55, 65 etc. als mittlere Klassenwerte zugrundegelegt.

	Mittlerer Samenertrag pro Individuum und mittlerer Fehler dieses Wertes
A Gl-Individuengruppe	$43{,}38 \pm 1{,}46$
A gl gl-Individuengruppe	$62{,}81 \pm 4{,}09$
a-Individuengruppe	$45{,}47 \pm 2{,}46$

Dass die beiden Individuengruppen *A Gl* und *A gl gl* eine statistisch sichere Differenz aufweisen ist aus diesen Zahlen unmittelbar zu ersehen. Eine Berechnung der Differenzen zwischen diesen drei Gruppen

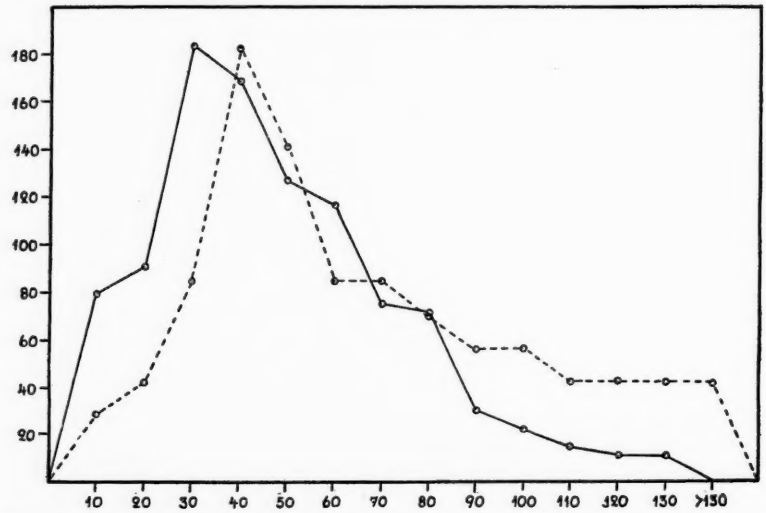

Fig. 1. Graphische Darstellung des Samenertrages der Individuen in F_2 von Kreuzung Nr. 224. Vollgezogene Kurve = *A Gl*-Individuen, gestrichelte = *A gl gl*-Individuen. Abszisse = Samenerträge in 10-Samenklassen, Ordinate = Individuenanzahl.

und den dazugehörigen mittleren Fehlern für diese sowie der Werte für $D/m_{Diff.}$ ergibt folgendes.

Differenz *A Gl* — *A gl gl* $= 19{,}43 \pm 4{,}34$; $D/m_{Diff.} = 4{,}48$.
» *A Gl* — *a* $= 2{,}09 \pm 3{,}49$; $D/m_{Diff.} = 0{,}60$.
» *A gl gl* — *a* $= 17{,}34 \pm 4{,}77$; $D/m_{Diff.} = 3{,}64$.

Aus diesen Zahlen ergibt sich, dass die Differenz in bezug auf den Samenertrag zwischen den beiden Gruppen *A Gl* und *A gl gl* statistisch als ganz sicher zu betrachten ist, denn die Differenz beträgt hier nicht weniger als ungefähr das $4\,^1/_2$-fache des mittleren Fehlers für dieselbe. Sehr schön wird diese Differenz zwischen den *A Gl*- und *A gl gl*-Indivi-

duengruppen auch durch die graphische Darstellung in Fig. 1 veranschaulicht. Die gestrichelte Kurve, die die *A gl gl*-Individuen repräsentiert, ist im Vergleich zur *A Gl*-Kurve (vollgezogen) deutlich nach rechts, nach höherem Samenertrag zu verschoben. Weniger stark ausgeprägt, aber doch noch immer statistisch sicher ist die Differenz zwischen der *A gl gl*-Gruppe und *a*-Gruppe; hier beträgt die Differenz mehr als das $3\,^1/_2$-fache ihres mittleren Fehlers. Zwischen der *A Gl*- und der *a*-Gruppe besteht dagegen kein sicherer Unterschied; dieser liegt ganz binnen der üblichen Fehlergrenzen.

Die Beobachtung, dass *gl gl*-Individuen sicher grössere Samenerträge geben als *Gl*-Individuen dürfte in zweierlei Weise erklärt werden können. Entweder wird diese Erscheinung durch Rezessivität in *gl* direkt verursacht oder es spaltet in dieser Kreuzung auch ein Gen mit beträchtlichem Einfluss auf den Samenertrag, das mit *gl* stärker gekoppelt ist. Es sei hier unmittelbar hervorgehoben, dass die Elternlinie L 22 (*gl gl*), wie zu erwarten, einen deutlich grösseren Samenertrag gibt als die andere Elternlinie, L 206 (*Gl Gl*). Mit Hinsicht auf den Samenertrag der Elternlinien liegt leider kein grösseres Material vor. In jedem der drei letzten Jahre wurden je drei der besten Pflanzen für sich geerntet. Eine Berechnung des mittleren Samenertrages auf Grund dieser neun Pflanzen für jede der Elternlinien ergibt folgendes:

TABELLE 3. *Der Zusammenhang zwischen Spaltung im Genpaar Gl—gl und dem Samenertrag.*

Gruppe	Ertrag in Anzahl Samen (10-Samenklassen)															Summe
	0—10	11—20	21—30	31—40	41—50	51—60	61—70	71—80	81—90	91—100	101—110	111—120	121—130	> 130		
A Gl-Individuen	21	24	49	45	34	31	20	18	8	6	4	3	3	—		266
A gl gl-Individuen	2	3	6	13	10	6	6	5	4	4	3	3	3	3		71
a $\frac{Gl}{gl}$-Individuen	5	14	15	14	14	13	11	9	5	2	2	1	—	—		105

L 22 58,33 Samen pro Pflanze
L 206 33,33 » » »

Was hier als Samenertrag bezeichnet wird, bezieht sich auf die Anzahl Samen pro Pflanze, nicht etwa auf das Gewicht. In letzterer Hinsicht wird nämlich der Unterschied zwischen den beiden Eltern von Kreuzung 224 durch das Samengewicht zu grösserem Teil wettgemacht. 100 Samen von L 22 wiegen nämlich durchschnittlich 19,5 g, von L 206 dagegen 26,5 g. Das ist ein Mehr von etwa 36 %, das jedenfalls mehr als die Hälfte der Differenz zwischen den beiden Elternlinien ausmacht. Der grösseren Differenz zwischen den Elternlinien ist überdies wegen der geringen Anzahl untersuchter Pflanzen (je 9) kein grösserer Wert beizulegen. Wie sich diese Beziehungen zwischen Anzahl Samen pro Pflanze und Samengewicht in F_2 gestalten ist nicht untersucht worden.

Da es sich in vorliegender Kreuzung um die Wirkung eines Gens handelt, das einen erheblichen Einfluss auf die Anzahl Samen pro Pflanze hat, die ihrerseits wiederum mit der Anzahl entwickelter Hülsen, Anzahl von Samenanlagen pro Hülse und dem Prozent solcher sich entwickelnder zusammenhängen dürfte, dünkt es mir wahrscheinlich, dass es sich nicht um eine ev. pleiotrope Wirkung von *gl*, sondern um den Effekt eines anderen mit *gl* gekoppelten Gens handelt. Die F_2 kann diese Frage nicht entscheiden. Eine weitere Generation wird jedoch hierauf voraussichtlich klare Antwort geben, denn in einer solchen sollen Familien auftreten, die in dem die Samenanzahl beeinflussenden Gen homozygot sind, und die daher in *Gl*—*gl* spalten werden ohne dass die *gl gl*-Individuen durch einen höheren Samenertrag charakterisiert werden.

In beiden Fällen, ob es sich nun um eine pleiotrope Wirkung von *gl* oder um ein anderes mit diesem gekoppeltes Gen handelt, ist für den Samenertrag der *aa*-Individuen in F_2 ein bestimmter Wert zu erwarten — wobei allerdings stillschweigend vorausgesetzt wird, dass nicht auch eine Koppelung mit *a* oder Koppelung eines weiteren Gens für Samenertrag mit *a* vorhanden ist. Die *aa*-Individuen sollen einen Ertrag geben, der gleich ist dem der *A Gl*-Individuengruppe, erhöht mit einem Viertel der Differenz zwischen dieser und der *A gl gl*-Individuengruppe, denn die *aa*-Individuen sollen zu drei Viertel aus *Gl*- und zu einem Viertel aus *gl gl*-Individuen bestehen. Eine Berechnung dieses erwarteten Wertes ergibt 48,24 Samen pro Pflanze. Gefunden wurden (siehe oben) 45,47 $\pm$2,46 und D/m beträgt für die Abweichung von der erwarteten Zahl nur 1,13. Die Übereinstimmung zwischen dem gefundenen und dem erwarteten Wert für den Samenertrag der *aa*-Pflanzen ist demnach

eine gute. Alle diese Erwägungen hinsichtlich des Samenertrages gelten natürlich, ganz gleichgültig wie sich die Dominanzverhältnisse für ein solches angenommenes, mit *gl* gekoppeltes Gen gestalten.

SUMMARY.

1. The effect of the gene *Gl*, which was advanced in 1928 by H. and O. TEDIN as a »new hypothetical factor», is studied in detail, the following results being obtained:

2. In its doubly recessive form, *gl gl*, the gene *Gl* causes a local ochre-yellow colouring of the seed-coat in *Pisum sativum* on the place of the radicle.

3. The gene *Gl* is very probably situated in the same chromosome as the *B* gene.

4. The occurrence of *gl gl* individuals as recessive mutants is shown in the F_4 and F_5 of a cross, which was studied with respect to segregation in 7 pairs of genes.

5. In conjunction with the segregation in the gene-pair *Gl—gl* a high degree of segregation was ascertained with respect to seed-setting, the high seed production being shown to be linked with *Gl*.

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INTRA- AND INTERSPECIFIC HYBRIDS OF PHLEUM PRATENSE AND P. ALPINUM

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(Preliminary report)

SINCE the summer of 1934 I have been working on some crosses between different species of *Phleum* at the Cyto-Genetic Department of Svalöf. Though the experiments have not been carried through farther than to F_1, I have, nevertheless, found them worth a brief description. No definite conclusions are of course possible without further investigations, but in many cases the data indicate at least some points.

MATERIAL.

The material was to a great extent received from the Forage Crop Department of Svalöf. Dr. N. SYLVÉN, head of this department, kindly helped me to select suitable plants from among his material of timothy grass, which was of great value to me. Since it is impossible to emasculate successfully the very small flowers of timothy, it is necessary to have male or self-sterile mother plants for the crosses. Among those plants I got from Dr. SYLVÉN, there were two practically male sterile biotypes of *Phleum nodosum* L., and one almost self-sterile biotype of *Phleum pratense* L.

P. nodosum L. is diploid with $2n = 14$, and is synonymous with the *P. pratense*, Group II, described by GREGOR and SANSOME (1930). The two plants I got of that type (Field Nos. 575 and 584) were sister plants and descendants of a plant from Skanör in the Province of Skåne, Sweden (SYLVÉN, 1929). They were practically male sterile, i. e. the anthers did not dehisce, though they contained some good pollen grains. One reason for the sterility may be the fact that the meiosis of the pollen mother cells was not quite regular. Very often one of the chromosome pairs showed asyndesis (Figs. 1 and 2). The fertility of the female gametes was also lowered, plant 584 especially gave very few seeds after open pollination.

P. pratense L. is hexaploid, having $2n = 42$ and corresponding to the *P. pratense*, Group I, described by GREGOR and SANSOME (1930).

The plant used in the crosses (Field No. 308) was of unknown origin (SYLVÉN, 1929). It was almost self-sterile, giving only a few seeds by self-fertilisation, and was consequently used with advantage as mother plant in the crosses.

At the Cyto-Genetic Department of Svalöf, Dr. A. MÜNTZING had two types of *P. alpinum* L. One of them was a tetraploid with $2n = 28$, obtained from Pajala and Pello in the Scandinavian mountains. It must be a type similar to that described by GREGOR and SANSOME as Scottish *P. alpinum*. The same type occurs, moreover, in the Alps. I received some plants from Dr. G. NILSSON-LEISSNER, which were obtained from Arosa, Switzerland. They were tetraploid and similar to the *P. alpinum* from Scandinavia. In this tetraploid *alpinum*, however, I did not find any male or self-sterile individuals which could be used as mother plants. The second type is diploid with $2n = 14$ (MÜNTZING, 1935). It originated from the Alps. This type must correspond to the *alpinum* type which GREGOR and SANSOME have described as Swedish *P. alpinum*. They received it from Dr. F. NILSSON, who has, however, informed me that the plants did not originally come from Sweden but from Germany. This type of *P. alpinum* was represented at Svalöf by only one biotype, which proved to be absolutely self-sterile. No seeds were obtained, though the plant has been under control for three years. The pollen seems to be quite normal when stained in an acetocarmin-glycerin preparation, and the meiosis of the pollen mother cells is regular. However, no seeds were ever obtained by using this pollen in a cross. It has been used to pollinate both *P. pratense* and *P. nodosum* and the last combination at least should have given seeds if the pollen is functional. The reciprocal cross (*P. alpinum* $\times$ *P. nodosum*) was very successful (see below).

These four types of the genus of *Phleum* now described are all very closely related. In ASCHERSON and GRÆBNER's Manual (1899), they are characterized as one species, *P. pratense*, which is divided into two subspecies, *P. vulgare* and *P. alpinum*, the former corresponding both to *P. pratense* L. and to *P. nodosum* L. and the latter to *P. alpinum* L. Besides these very closely related types of the *pratense* group I also used some other species of *Phleum* in the crosses. Those are *P. Michelii*, *P. Boehmeri*, and *P. ambiguum*. But all the crosses between these species and those of the *pratense* group failed, while, on the other hand, the crosses between the different types within the *pratense* group were succesful.

METHODS.

The plants which were to be used for the crosses were planted in pots and kept in the greenhouse. They did not, however, always flower at the same time. In order to attain that, those which flowered earlier were placed in a cool room, having a temperature of about + 3°. Some of the plants were kept there for only a short time, some for a long time, up to three weeks. This treatment did not seem to injure them at all. In this manner I had flowering *P. alpinum* plants for a long time in the early summer. The crosses were made in isolation boxes in the greenhouse, where the two plants were placed and kept till they had ceased flowering.

In order to determine the chromosome number of the plants, root tips were fixed in diluted chromacetic formalin, and the slides stained with gentian violet. To study the meiosis of the pollen mother cells spikelets were fixed in the same fixative with pre-fixation in Carnoy. These sections were also stained with gentian violet.

THE CROSSES.

P. NODOSUM (2n = 14) × P. PRATENSE (2n = 42).

This cross was made in the summer of 1934. It was surprisingly successful and all the hybrids looked almost like the father plant *P. pratense*. When the chromosome number of the hybrid was determined it was found to be not 28, as expected, but 35, and some of the plants (about 10 per cent.) had 49 chromosomes (see Table 1). Two biotypes of *P. nodosum* were used (Nos. 575 and 584) and both behaved in the same way. This cannot be explained in any other way than by assuming the female gametes of the mother plant to be unreduced or to have twice the somatic number of chromosomes. To find out if these two biotypes of *P. nodosum* produced also normal reduced gametes, progeny was procured from them in the following ways: firstly it was taken from a cross between these plants and another normal *nodosum* plant, secondly from seeds after open pollination.

In the first cross 20 plants were investigated, 10 from each mother plant. They were all *nodosum*-like and all of them, except three plants, had 14 chromosomes. Among these three plants two were triploid, one from each mother plant, while another plant among the offspring of plant 584 had 17 chromosomes. The result proves that there are plenty of reduced normal gametes formed. Moreover, it shows that the plants may occasionally form gametes which have a chromosome num-

ber intermediate between 7 and 14, in this case 10. This is not surprising as the meiosis of the pollen mother cells is not regular (see above). The triploids which were found in the progeny have probably arisen from the fusion of an unreduced female gamete and a reduced normal male gamete.

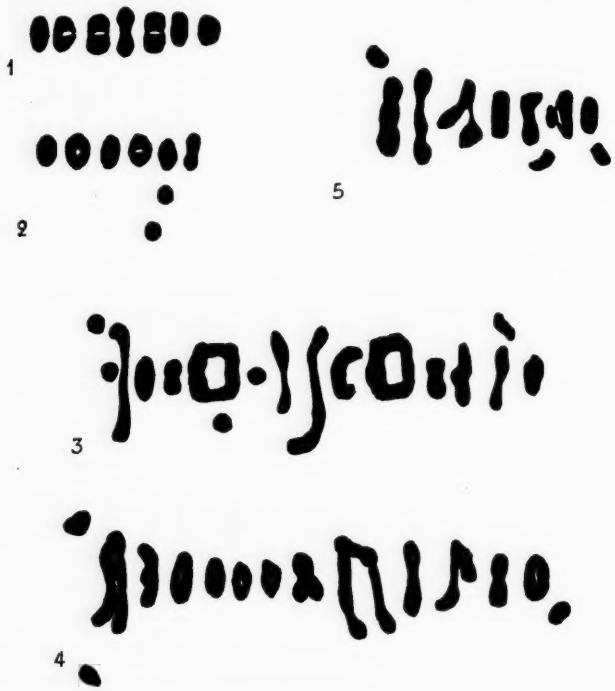
TABLE 1. *The result of crosses between different Phleum species of the pratense group.*

Cross combination	Spikes pollinated	Seeds obtained	Seeds planted	Hybrid plants obtained	Non-hybrid plants obtained	Hybrid seeds per spike	2n of hybrid plants
P. nod., 584, (14) × *P. prat.*(42)	5	55	50	48	0	11,0	35,49
P. nod., 575, (14) × *P. prat.*(42)	5	126	15	14	0	25,2	35,49
Dipl. *P. alp.* (14) × *P. prat.*(42)	2	2	2	2	0	1,0	35,49
P. prat. (42) × *P. nod.* (14) ...	35	3	3	2	1	0,06	28
P. prat. (42) × tetrapl. *P. alp.* (28)	17	21	21	3	18	0,18	35
Dipl. *P. alp.* (14) × *P. nod.* (14)	1	65	20	16	0	65,0	14
Dipl. *P. alp.* (14) × *P. nod.*, 575 (14)	1	13	10	7	0	13,0	14
Dipl. *P. alp.* (14) × tetrapl. *P. alp.* (28).....	2	170	20	18	0	85,0	21
P. nod., 584, (14) × tetrapl. *P. alp.* (28).....	3	10	10	9	0	3,3	21
P. nod., 575, (14) × tetrapl. *P. alp.* (28).....	3	85	45	31	8	22,5	21

The second progeny, which was taken from the plants after open pollination, was of mixed appearance, as was to be expected. Some of the plants were of *nodosum* type and had 14 chromosomes, the others of *pratense* type with 35 and 49 chromosomes respectively. No triploids were found. In the offspring of plant 584, 50 plants were investigated, 10 per cent. of which were *nodosum*-like, and in the progeny of plant 575, 20 plants were examined, 45 per cent. of which were of the *nodosum* type.

The cross *P. nodosum* × *P. pratense* only gave plants with 35 and 49 chromosomes. The mother plant must, however, have produced at least three different kinds of female gametes; normally reduced gametes with 7 chromosomes, unreduced with 14 chromosomes, and gametes with 28 chromosomes, i. e. twice the somatic number of the plant. When these different kinds of gametes were fertilized by pollen

from *P. pratense* with a gametic chromosome number of 21, all the different zygotes formed were probably not equally viable (cf. MÜNTZING, 1936, p. 330). Those formed by fertilisation of the normally reduced gametes must have died, whereas the others were more viable. The differ-



Figs. 1—2. *P. nodosum* (plant 584). Heterotypic metaphase of the pollen mother cells (separately drawn); Fig. 1 shows a regular metaphase, Fig. 2 a metaphase when one chromosome pair shows asyndesis. — Figs. 3—4. *P. nodosum* × *P. pratense* (with 35 chromosomes). Heterotypic metaphase of the pollen mother cells (separately drawn); Fig. 3 shows meiosis with $2_{IV} + 2_{III} + 8_{II} + 5_{I}$, Fig. 4 with $2_{V} + 2_{III} + 8_{II} + 3_{I}$. — Fig. 5. Triploid *P. nodosum* ($2n = 21$). Heterotypic metaphase of the pollen mother cells (separately drawn); the figure shows a metaphase with $4_{III} + 3_{II} + 3_{I}$. — Magnification: Figs. 1, 2, 5, ca. × 2500; Figs. 3, 4, ca. × 3000.

ence in chromosome number between male and female gametes is in the first case 14, but in the latter only 7. The reciprocal cross was nevertheless successful, in spite of the great difference in the number of chromosomes, and gave a hybrid with $2n = 28$ (see below). It is however very often easier to make a cross in one direction than in another, and fre-

quently it is much easier to make a cross with the maternal plant with a higher number of chromosomes than in the opposite direction.

Both the hybrids possessing 35 and 49 chromosomes were impossible to distinguish from *P. pratense* and they were very similar to each other. Those with 49 chromosomes seemed, however, to have fewer sprouts, though they were harder and more rigid. Both of them had good male and female fertility. The pollen fertility was between 80 per cent. and 95 per cent. good pollen, or about the same as in *P. pratense*. In the meiosis of the pollen mother cells of the hybrid with 35 chromosomes different configurations were formed. There were univalents, bivalents, and multivalents of different kinds up to associations of five. As fixation was good it was possible to make complete analysis of several first metaphase groups (Figs. 3 and 4). The quadrivalents were very often ring-shaped. The univalents were never more than seven in number, usually less numerous. Fixation of meiosis of the hybrid with 49 chromosomes was not very good and the chromosomes were so numerous that it was impossible to distinguish completely all the chromosomes in the metaphase groups. They contained, however, besides univalents and bivalents plenty of multivalents.

As mentioned above, the triploid *P. nodosum* plants were similar to ordinary *P. nodosum*. They were male sterile, as the anthers did not dehisce. The female gametes functioned in the cross to *P. pratense*, which yielded several good seeds, whereas the cross to *P. nodosum* gave only one rather bad seed, which probably will not germinate. As it may be expected that amongst all the different gametes formed by the triploid *P. nodosum* the unreduced female gametes with 21 chromosomes are those which will give the most viable zygotes with pollen from *P. pratense*, most of this progeny will probably be hexaploid. The meiosis of the pollen mother cells was irregular, and univalents, bivalents, and trivalents were observed (Fig. 5). The total number of bivalents and trivalents in the metaphase groups was seven in all the 20 cells investigated, except two. One of latter had eight, the other only six. The univalents were scattered in the cells.

DIPLOID P. ALPINUM ($2n = 14$) $\times$ P. PRATENSE ($2n = 42$).

This cross was made in the summer of 1935 and gave very few seeds. Only two seeds, and from them two plants, were obtained after pollination of two spikes (see Table 1). The chromosome numbers of the two hybrids obtained were 35 and 49 respectively. This is not very surprising, considering the result of the cross *P. nodosum* $\times$ *P. pratense*.

But the meiosis of the pollen mother cells of the mother plant, the diploid *P. alpinum*, was here absolutely regular and the pollen good (see above). In other words, the plant seemed to be normal, which was not the case with the *P. nodosum* plants. And yet it was able to produce female gametes which were unreduced or contained twice the somatic number of chromosomes, though in a very low frequency. Those few gametes functioned in the cross with *P. pratense*. That this diploid *P. alpinum* plant had plenty of normal reduced gametes is shown by the crosses between this plant and *P. nodosum* and tetraploid *P. alpinum* (see below).

The morphological differences between the two hybrid plants were rather striking. The plant with 49 chromosomes had fewer but more robust sprouts, and the leaves were longer. Both of them were, however, similar to the mother plant. This circumstance is in contrast to the result of the earlier cross *P. nodosum* × *P. pratense*, where the hybrids were most similar to the father plant. No further investigation of the hybrids was possible, as neither of the plants flowered this summer.

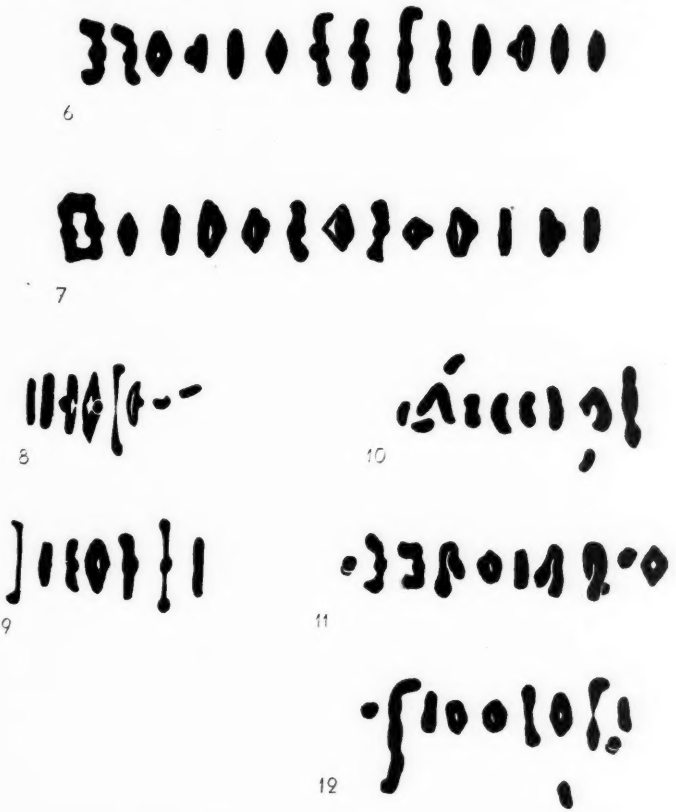
P. PRATENSE (2n = 42) × P. NODOSUM (2n = 14).

As mentioned above, this cross succeeded although with difficulty. Only two hybrid plants were obtained from 35 pollinated spikes (see Table 1). The hybrids had 28 chromosomes and were of an intermediate habit. Both male and female fertility were good. One plant had 70 per cent. and the other 90 per cent. good pollen. The former seemed to be absolutely self-sterile and the latter had a very poor self-fertility. But their female fertility, when the plants were crossed with each other, was good. The meiosis of the pollen mother cells was quite regular. The chromosomes formed 14 bivalents, and in a few cells one ring-shaped quadrivalent was observed (Figs. 6 and 7). Thus one genome of *P. pratense* must be homologous to the genome of *P. nodosum*, and the other two genomes of *P. pratense* homologous to each other and probably also to the first mentioned.

P. PRATENSE (2n = 42) × TETRAPLOID P. ALPINUM (2n = 28).

This cross was made in the summer of 1935. It was successful, but after some difficulty. Only three hybrid plants were obtained from 17 pollinated spikes (see Table 1). These hybrids had 35 chromosomes. They were most similar to *P. pratense*, although they had some of the *alpinum* characters. The pollen fertility of two of the plants was good

(90 per cent.), but the third plant had very poor pollen and the anthers did not dehisce. The meiosis of the pollen mother cells was badly



Figs. 6—7. *P. pratense* × *P. nodosum*. Heterotypic metaphase of the pollen mother cells (separately drawn); Fig. 6 shows meiosis with 14 bivalents, Fig. 7 meiosis with $12_{II} + 1_{IV}$. — Figs. 8—9. Diploid *P. alpinum* × *P. nodosum*. Heterotypic metaphase of the pollen mother cells (separately drawn); Fig. 8 a metaphase in which one of the chromosome pairs shows asyndesis, Fig. 9 shows a regular metaphase. — Figs. 10—12. *P. nodosum* × tetraploid *P. alpinum*. Heterotypic metaphase of the pollen mother cells (separately drawn); Fig. 10 shows a metaphase with $3_{III} + 4_{II} + 4_{I}$, Fig. 11 metaphase with $3_{III} + 5_{II} + 2_{I}$, Fig. 12 a metaphase with $1_{IV} + 7_{II} + 3_{I}$. — Magnification: Figs. 8, 10, × 2500; Figs. 6, 7, 9, 11, 12, × 3000.

fixed. A complete analysis of the chromosome complements in the cells was not possible. There were univalents, bivalents, and multi-

valents of different kinds, chains and ring quadrivalents, but they seemed to be less numerous than in the pollen mother cells of the hybrid *P. nodosum* × *P. pratense*, with 35 chromosomes.

DIPLOID P. ALPINUM (2n = 14) × P. NODOSUM (2n = 14).

This cross was made in the summer of 1935 and was very successful (see Table 1). As expected, the hybrids were diploid with 2n = 14. They were bigger than either of the parents and looked almost like *P. pratense*. In the meiosis of the pollen mother cells rod and ring-shaped bivalents were observed. Only in very few cells did one of the chromosome pairs show asyndesis (Figs. 8 and 9). The pollen fertility was rather good, but it was better in one case than in the other. In the two cases different biotypes were used as the male parent. With the former the pollen fertility was about 50 per cent. and the anthers dehisced. With the other the fertility was only 10—20 per cent. and the anthers did not dehisce. The size of the pollen grains varied considerably, but the larger grains were on the average not greater in size than the pollen grains of the parents. The female fertility was good and the plants gave more or less seeds after cross- and self-fertilisation. They were also crossed with *P. nodosum* and with *P. pratense*. Both these crosses gave seeds, though the seeds from the latter might be the product of self-fertilisation.

DIPLOID P. ALPINUM (2n = 14) × TETRAPLOID P. ALPINUM (2n = 28).

This cross was made in the summer of 1934 and was also very successful (see Table 1). The hybrids were triploid with 2n = 21, larger than either of the parents, and of an intermediate type. They were completely male sterile and also the female gametes did not seem to function. No seeds were obtained when the hybrid was back-crossed to both of the parents. The cross with *P. pratense* gave the same negative result. That cross was tried in order to find out if the hybrid had unreduced female gametes capable of functioning. The meiosis of the pollen mother cells was badly fixed, but bivalents, trivalents and univalents were observed in the metaphase groups.

P. NODOSUM (2n = 14) × TETRAPLOID P. ALPINUM (2n = 28).

This cross was made in the summer of 1935 and also gave a very good result (see Table 1). These hybrids were triploid with 2n = 21. The number of chromosomes was determined on 34 hybrid plants and

all of them were triploid, none tetraploid. That is rather surprising, as the mother plants used were the same as in the cross *P. nodosum* × *P. pratense*, and thus formed unreduced female gametes (see above). These unreduced gametes functioned not only with pollen from the hexaploid *P. pratense* but also with pollen from the diploid *P. nodosum*.

The hybrids of this cross were of an intermediate type, and completely male sterile. It was not possible to control the female fertility this summer as the plants flowered very late in the autumn. According to GREGOR and SANSOME (1930) their hybrids of the same kind were partly fertile, and they succeeded in getting progeny of them. In the meiosis of the pollen mother cells univalents, bivalents, and trivalents were observed, and in a few cases also a quadrivalent (Figs. 10—12). The total number of bivalents and trivalents was seven or eight. In 37 per cent. of the 30 cells counted the number was eight. Then two of the univalents must be able to pair, which indicates that two of the chromosomes of the same genome are partially homologous. The same fact reveals the quadrivalent. These partially homologous chromosomes probably do not belong to the genome of *P. nodosum*, as such a pairing in this genome does not seem to occur according to the associations formed by the chromosomes in the meiosis of the triploid *P. nodosum*.

As is evident from Table 1, the success of the crosses was quite variable. But the reason of the differences in result is not always the same. In the crosses *P. nodosum* × *P. pratense* and diploid *P. alpinum* × *P. pratense* the result is due to the presence of female gametes with the somatic or twice the somatic number of chromosomes in the mother plants. The two biotypes of *P. nodosum* formed plenty of them, whereas the biotype of *P. alpinum* seems to form only a very few. The difference in the success of the other crosses must depend on other reasons. When the difference in chromosome number is small, as in the crosses diploid × diploid and diploid × tetraploid, the result was, however, better than when the difference was greater, as in the cross *P. pratense* × *P. nodosum*.

Not many cyto-genetic investigations on the genus of *Phleum* have been carried out. The most comprehensive investigation was made by GREGOR and SANSOME (1930). In their crosses they used the same species of *Phleum* as were used here. They did not, however, succeed in crossing *P. pratense* with *P. nodosum* (which they called *P. pratense* Group I and Group II). But they succeeded in crossing both these types

with tetraploid *P. alpinum*. The cross *P. nodosum* (2n = 14) × *P. alpinum* (2n = 28) gave a hybrid with 21 chromosomes. These plants were partially fertile and in the progeny some plants with 42 chromosomes were obtained. According to the authors these plants probably arose from the fusion of gametes having the somatic (3n) number of chromosomes. But as the seeds were obtained from unprotected flowers in the experimental field they might have been a cross between unreduced female gametes of the hybrids and pollen from *P. pratense* (2n = 42). One of these hexaploid plants was, however, completely fertile and easily crossed with *P. pratense* and many of the hybrids obtained were both male and female fertile (GREGOR, 1931). The triploid F_1 hybrid was also back-crossed to the tetraploid *P. alpinum* parent and then gave plants with varying numbers of chromosomes.

GREGOR and SANSOME got only one hybrid with 35 chromosomes from the cross *P. pratense* (2n = 42) × *P. alpinum* (2n = 28). This was completely sterile and they did not succeed in getting progeny from it. The same was the case with the diploid hybrid between *P. alpinum* (2n = 14) × *P. nodosum* (2n = 14) (GREGOR, 1931). These two kinds of hybrids are not, however, necessarily sterile. They can also be more or less fertile, as has been described in this report.

Cyto-genetic studies on species of *Phleum* were also undertaken by MÜNTZING (1935). The author found some timothy plants with 35 and 36 chromosomes in the material of the Forage Crop Department of Svalöf. They looked almost like *P. pratense*. He assumed that they had arisen through spontaneous hybridisation between *P. pratense* and *P. nodosum*, and that the plants found were products of back-crosses between the primary hybrid and *P. pratense*. They were male and female fertile and showed irregular meiosis in their pollen mother cells. That these plants were the offspring from a hybrid of *P. nodosum* × *P. pratense* is evident, but in the light of the results described in the present paper it is not necessary to presume a back-cross to *P. pratense* to explain the number of their chromosomes.

The four types of *Phleum*, which were used in the crosses, are very closely related, and may be characterized as the *pratense* group of the genus of *Phleum*. It is possible to cross them with each other, and the cytological investigation proves that their genomes are more or less homologous. But still they are distinctly different species with different numbers of chromosomes, which is the reason why it is not always easy to cross them. The genomes of *P. nodosum* and *P. pratense* must be very similar. The hybrids between these species, having 28 chromo-

somes, were morphologically of an intermediate type and had a regular meiosis with a few quadrivalents. The hybrids with 35 and 49 chromosomes were almost impossible to distinguish from true *P. pratense* and at their meiosis large numbers of multivalents were present. These facts strongly indicate that the difference between *P. nodosum* and *P. pratense* depends more upon the quantity than the quality of the chromosomes. But still there are no multivalents present in the meiosis of the pollen mother cells of *P. pratense*.

The hybrids between diploid *P. alpinum* and *P. pratense* with 35 and 49 chromosomes, on the other hand, were mostly similar to the mother plant, the diploid *P. alpinum*. That proves the fact that the difference here between diploid *P. alpinum* and *P. pratense* depends upon both the quality and the quantity of the chromosomes. But the dissimilarity of the genomes is not so great as to prevent the hybrids between *P. nodosum* and diploid *P. alpinum* from having an almost regular meiosis.

SUMMARY.

The *pratense* group of the genus *Phleum* contains four different types which have distinct morphological and genetic differences. These four types are *P. pratense* L. ($2n = 42$), *P. nodosum* L. ($2n = 14$), *P. alpinum* L. ($2n = 28$), and *P. alpinum* L. ($2n = 14$). These different species of *Phleum* may be crossed with each other, some readily, some with difficulty.

In the meiosis of the pollen mother cells of the hybrids the genomes from the different types are shown to be more or less homologous. Hybrids with an even number of genomes (diploid *P. alpinum* $\times$ *P. nodosum* and *P. pratense* $\times$ *P. nodosum*) have a regular meiosis in their pollen mother cells and are mostly fertile. The triploid hybrids are male sterile while hybrids with a higher, uneven number of genomes, with 35 and 49 chromosomes, are usually both male and female fertile. The triploids form univalents, bivalents and trivalents, and hybrids with 35 and 49 chromosomes also form multivalents of higher degrees.

I wish to express my thanks to all those who have helped me and facilitated my work at Svalöf. I wish especially to thank Professor H. NILSSON-EHLE and Dr. A. MÜNTZING for permitting me to do my work at the Cyto-Genetic Department of Svalöf and I am greatly in-

debted to Dr. MÜNTZING for all the aid he has given me in my work. I also want to thank Dr. N. SYLVÉN for the use of plants of his material and much valuable information. Finally I am much obliged to Miss M. PALM for looking after my plants during my absence from Svalöf.

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CYTOLOGICAL STUDIES IN THE ALLIUM PANICULATUM GROUP

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INTRODUCTION.

IN the genus *Allium* there occasionally occur in nature forms with different chromosome numbers within the same species or within a group of closely related species. In the majority of cases these then form a polyploid series, the most common case being the occurrence of diploids and tetraploids. However, triploids also occur in nature and can by means of vegetative propagation acquire a wide geographical distribution. In the *Allium* group, which is the subject of treatment in the present paper, the *paniculatum* group, I have up to now found only euploid forms in nature, viz. 3 diploid species, *A. paniculatum*, *A. pulchellum* and *A. flavum*, 1 triploid species, *A. carinatum*, and 1 tetraploid, *A. oleraceum*. The somatic chromosome numbers of these species are multiples of 8, the different forms thus having $2n = 16$, 24 and 32 respectively. Morphologically, these species are closely related and clearly distinguished from other species of *Allium*, and therefore it is practical to combine them into one unit, the *paniculatum* group.

The cytology of *A. carinatum* and *A. oleraceum* has been described in an earlier paper (LEVAN, 1933). Mainly on cytological grounds, chromosomal morphology, meiotic pairing, etc., I put forward the suggestion that these two polyploid species were links in one and the same polyploid series, the diploid members of which were to be found among the closely allied diploid species *A. paniculatum*, *A. pulchellum* and *A. flavum*. I emphasized, however, that experimental results would be necessary before this hypothesis could be anything but a conjecture. During the following years I have also been carrying on experiments for the purpose of testing this hypothesis. I have now succeeded in obtaining definitive proofs that the hypothesis, if not entirely wrong, is nevertheless insufficient.

In a short preliminary paper (LEVAN, 1936 c) I have already published the gist of these investigations: *A. paniculatum* has by

chromosome doubling given rise to a typical form of *A. oleraceum*, and from the triploid *A. carinatum* diploid forms have been extracted agreeing with *A. pulchellum*. Tetraploids or quasi-tetraploids have been formed by *carinatum* genomes alone or by combined *carinatum—pulchellum* genomes and they are exactly like *A. carinatum* and do not show any transgression in the direction of *A. oleraceum*. Consequently that part of my hypothesis, which assumed that these species belonged to the *same* polyploid series, fails. Instead we must now reckon with 2 polyploid series in the *paniculatum* group, in which the species formation in nature has proceeded along parallel lines. In one series the formation of species has reached a definitive stage, a tetraploid form, which even if it is induced to set seed has a prospect of remaining constant. In the other series with the triploid *carinatum*, on the contrary, a more unstable condition is brought about. As long as propagation takes place only vegetatively the species will remain constant, but on the first opportunity of sexual propagation it swings over to the diploid or tetraploid state of equilibrium, which really occurred in the experiments, an account of which will be given below.

First a brief historical outline will be given of the views of taxonomical science on the species in the *paniculatum* group. This will be followed by a description of my material of the various species of *paniculatum* which were first cultivated at Lund (up to 1932) and afterwards at Hilleshög, Landskrona. This material has partly, although unfortunately to a small extent, originated from natural habitats, partly obtained by seed exchange from different botanical gardens. A description will then be given of the crossing experiments carried out and the forms arisen by means thereof. Finally, an account will be given of the cytology of the different forms, special attention being devoted to the chromosome pairing at meiosis in diploids and polyploids.

My thanks are due to Dr. ARTUR HÅKANSSON for advice and criticism and to Miss MAJ SJÖGREN for valuable technical assistance.

I. MORPHOLOGICAL SECTION.

1. HISTORICAL-TAXONOMICAL ORIENTATION.

The species of *Allium* dealt with in this paper, which I have brought together under the name of the *paniculatum* group, belong to REGEL's *Allium*, Section IV *Macrospatha*. This section is characterized by the absence of rhizomes, by tapering spathes and by stamens with-

out lateral lobes. The *paniculatum* group is distinguished from other *Macrospatha* by the bushy inflorescence, in which the pedicels are of unequal length.

The species of the *paniculatum* group form a very natural unit with a generally speaking similar morphology. The following description may be regarded as common for all species of *paniculatum*: Bulbs are round to tapering ovate, external bulb scales at first white, then becoming darker. The scape is upright or arched ascendent, leafy about to middle. The leaves are narrowly linear flat or round, the veins and margins of the leaves are often scabrous. The spathe is green, leaf-like, tapers to a sharp point, which is mostly several times longer than the inflorescence. The spathe splits into 2 lobes which are drawn straight down during flowering. The inflorescence has the same appearance in all species, lax, few-flowered or many-flowered. The pedicels are unequal in length, often several times longer than the flowers. In the budding and fruiting stages the flowers are erect, during the flowering period, on the other hand, they are pendulous. The flowers are infundibular to campanulate or semi-spherical. The perianth leaves are tapering—elliptic ovate, the 3 outer leaves often somewhat keeled. At the tip they are obtuse or pointed, occasionally furnished with a mucronate tip. The ovary is ovate, the style is at first short, later protruding, the stigma is punctiform.

Thus there is no difficulty in finding many similarities between the different species of the *paniculatum* group. The accompanying photographs will furnish a still plainer picture of the general appearance that these species have in common. Difficulties arise however when we wish to differentiate the various forms in the group from each other. For practical reasons I have below chosen a division of the species in which the species have been made as wide as possible. The group has been divided into species without bulbils and species with bulbils. Both these divisions thus comprise species with short and with long filaments. The synopsis of the species will be as follows:

1. Inflorescences without bulbils (diploid species)
   - A. Stamens shorter than the perianth leaves or equally long ..... 1. *paniculatum*
   - B. Stamens twice as long as the perianth leaves
     - a. Red flowers ..... 2. *pulchellum*
     - b. Yellow flowers ..... 3. *flavum*

2. Inflorescences with bulbils (polyploid species)

A. Stamens shorter than or as long as the perianth leaves ..... 4. *oleraceum*

B. Stamens twice as long as the perianth leaves .. 5. *carinatum*

Although this division of the species, like all schematic classifications, is to a certain extent artificial, I nevertheless believe that it reflects a deeper causal relation, in that the 5 species distinguished here form in a certain measure the state of equilibrium that the gene assortment of the *paniculatum* group is inclined to occupy in nature. My cultural experiments with these species show, for instance, that in addition to the length of the stamens, which separates *paniculatum*—*oleraceum* from the other species, there are also a number of other distinguishing characters, often difficult to define, which show that a division based on such an arbitrary character as the length of the stamens has at the same time supplied a comprehensive and natural division of the present material. This fact, which may seem to be a chance occurrence but which is really due to the surprisingly keen eyes of taxonomists for essentials, will find its explanation and its real background in the experiments described in the present paper. These will show that the division made on the basis of the length of the stamens lies deeper than purely morphological similarities and dissimilarities, in fact it has a genic and phylogenetic foundation.

Below I shall examine the different *paniculatum* species from a historical—taxonomical point of view, and mention their more important varieties and forms, such as they are met with in nature.

A. ALLIUM PANICULATUM.

This is undoubtedly the species which has the greatest number of forms in the group, and its various forms have been estimated quite differently at different times. Thus LINNÉ (1753) distinguishes 2 species, *pallens* and *paniculatum*, the former having light-coloured flowers, the latter having more or less red-coloured flowers. WILLDENOW (1799) describes the flowers of these two species as follows, *pallens*: »Corolla campanulata, truncata, alba, petalis obovatis, obtusissimis, erectis, concavis, carina viridi, stamina simplicia, longitudine petalorum, flores mox a florescentia pendent», and *paniculatum*: »Flores pedunculis capillaribus, longissimis, laxis. Petala purpurea, obovata, longitudine staminum». Gradually more and more closely related forms are being separated as species, the decisive feature being chiefly differences in the colour and the shape of the perianth leaves. Several such forms are reproduced in REICHENBACH's Icones (1848): 1064. *fuscum* W. K. perianthio obtuso, 1065. *intermedium* DEC. perianthii partitionibus obovatis, 1066. *praescissum* RCHB. perianthio cylindrico prismatico retuso, 1068. *longispathum* RED. perianthii parti-

tionibus lanceolatis. On the other hand, the author's *paniculatum* and *pallens* are pure *pulchellum* forms. LEDEBOUR (1853) adds a few-flowered *paniculatum* form with short pedicels, *β macilentum* from Russia.

In REGEL's monograph (1875) most of these described species are referred to the *paniculatum* species. He retains, however, as independent species *A. fuscum* W. K. (folia late linearia plana) and *A. stamineum*, *A. sipyleum* and *A. Achajium* proposed by BOISSIER, all of which, however, are closely related to *paniculatum*. REGEL divides the *paniculatum* species into 7 subspecies: *α typicum* (glaberrimum, umbella multiflora, pedicellis florem pluries superantibus), *β caucasicum* (foliis margine ad nervos vaginarum minute denticulato-scabriusculis, umbella pluri-multiflora, pedicelli omnes erecti v. singuli nutantes v. rarius diffusi), *γ rupestre* (glabrum, floribus minus numerosis, pedicellis minus inaequalibus, florem paullo-duplo superantibus), consequently LEDEBOUR's *macilentum* belongs here, *δ pallens* (spatha umbellam densiorem subaequante v. usque duplo superante, sepalis albidis v. pallide carneis v. virescentibus v. rubello tinctis, saepissime nervo medio viridi v. rubro notatis), *ε longispathum* (spatha umbellam 3-pluries superante, sepalis viridibus v. lurido-rubescentibus v. sordide albidis, viridi v. rubro tinctis), thus REGEL places all *paniculatum* forms not having bright rose or crimson flowers under *δ* and *ε*, *ζ tenuiflorum* (sepalis lineari-oblongis subacutis roseis v. carneis), *η montanum* (glabrum, caule humile, umbella pauci-pluriflora, sepala oblonga, obtusa v. subacuta), *ϑ brevicaule* (caule nano, foliis vaginisque ad nervos vaginesque scabridis, umbella 10—20 flora). REGEL emphasizes the great variation within the species: »Wollte man . . . nach dem Vorhandensein eines kleinen Zahnes im Winkel zwischen dem Grunde der Staubfäden, nach der Länge des Griffels, ob der Fruchtknoten gestielt oder nicht, ob die Blüthenblättchen etwas breiter oder schmäler, ob solche vorn ausgerandet, spitz oder in eine kurze Spitze vorgezogen, ob Stengel niedrig oder hoch, die Dolde arm- oder reichblumig, Scheidenblättchen wenig oder viel länger als Dolde, dann durch die Blüthenfarbe und ob Blätter kahl oder schärflich etc., diese Art theilen, dann müssten nicht bloss alle obigen Formen als Arten angenommen werden, sondern es müsste auch noch eine ganze Reihe neuer Arten aufgestellt werden». Something along these lines was attempted by BOISSIER (1884) in his Flora orientalis, when he distinguished from among the *Macrospatha* species furnished with short stamens not less than 19 species. In GANDOGER's Conspectus (1910) we can obtain a survey of the number of species that has been described in *paniculatum*. For Europe alone this list includes 29 species under *A. paniculatum*.

KOCH's Synopsis, third edition (1907) divides *paniculatum* into 2 subspecies according to the shape of the perianth leaves: *obtusiflorum* and *tenuiflorum* (perianth leaves »stumpf» and »spitz» respectively). The former subspecies is again subdivided into *α typicum* RGL., *β pallens* L. (= *Coppoleri*) and *γ longispathum* DELAR. ASCHERSON and GRÆBNER (1905) give a good description of *paniculatum*. They call attention to the multiformity of the species: »Eine ausserordentlich veränderliche Art, deren Formen sehr schwer zu bewerthen sind». The species is first divided into 2 subspecies, *intermedium* and *tenuiflorum*, the latter corresponding to REGEL's *ζ* form and, like that, is characterized by »Perigonblätter länglich-linealisch bis lanzettlich, etwas allmählich in eine scharfe Stachelspitze zugespitzt». The subspecies *intermedium* is then divided according to the formation of the spathous leaves: I. Spathe shorter (at most 1,5 times as long as the inflorescence), thinner and more flaccid, and II. Spathe several times longer than the inflorescence, rigid. To the

former division belong forms with narrow linear leaves and forms with flat leaves at least 3 mm. in width. The former are *typicum*, which is characterized by having a vigorous growth, being many-flowered, the flowers small to medium size, perianth more or less bright scarlet (especially small-flowered is the form *parviflorum* BOVÉ), and *pallens*, which is distinguished by less vigorous growth, an inflorescence that appears to be denser owing to the shorter pedicels, yellow to white perianth leaves (this is therefore LINNÉ's species *pallens*). »Als Art kann diese (*pallens*) Rasse unmöglich aufgeführt werden, da sie vom Typus ausser durch unwesentliche Merkmale fast nur durch die Farbe der Blüthe abweicht». A form belonging to *pallens* is *marginatum*, the leaves of which are furnished with reddish brown streaks and which has white petals. Under I should also be classed *fuscum*, which has flat leaves and obtuse perianth leaves, as a rule, with brown median streaks. Division II, which has long, rigid spathous leaves, includes *longispathum* (which corresponds to REICHENBACH's *intermedium*). This form is of special interest, as it was once regarded as a bulbil-free form of *oleraceum* (KERNER, 1878, cited after ASCHERSON and GRÆBNER 1905). Certainly it also resembles very much the tall forms of *oleraceum*, which often have long, rigid and green spathous leaves. The form of *oleraceum* synthetized by me was distinguished by just these features, and it is perhaps more than a mere chance that a taxonomist wanted to refer this form of *paniculatum* to *oleraceum*.

A similar association between *paniculatum* and *oleraceum* is found in ROUY (1910), who divides *paniculatum* into *α typicum* RGL., *β micranthum* NOB. (*parviflorum* BOVÉ), *γ intermedium* NOB. and *δ longispathum* RGL. In this case it is the variety *intermedium* that is considered to occur occasionally furnished with bulbils: »ombelle souvant bulbillifère». Whether diploid forms of *paniculatum* with bulbils really occur in nature I am unable to say. The most probable thing is that an *oleraceum* type exhibited such a close habitual similarity to *paniculatum* forms growing in the vicinity that the author was tempted to refer them to the same species. I continually see examples of this phenomenon in my cultures, a phenomenon which is of course explained by the character of the *oleraceum* species of being an autotetraploid *paniculatum*.

A. paniculatum has the following geographical distribution: France, Iberian Peninsula, Italy and the islands, the Balkan Peninsula, central and southern Russia, temperate Asia, North Africa, the Canary Islands, Madeira.

B. ALLIUM PULCHELLUM.

The species of *A. pulchellum*, which is characterized by the absence of bulbils in the inflorescences, and by its red flower-colour and long stamens, is very closely related to *flavum* and *carinatum*, with which it is autogenomatic (see p. 363 of this paper). Different authors have classified it in various ways, some have referred it to *A. flavum*, some have made it a subspecies of *A. carinatum*. It was first raised to the rank of a species by DON in 1826. In the same year MERTENS and KOCH (1826) write under *A. carinatum*: ». . . aber von einer roth blühenden Abart des *A. flavum*, deren MARSCH. BIEBERSTEIN suppl. 258 gedenkt (dem *A. paniculatum* DECAND.) würde sich das *A. carinatum* im trocknen Zustande bloss durch die Zwiebelchen in der Dolde, und wenn es mit und ohne Zwiebelchen in der Dolde abändern sollte, wie wir vermuthen, gar nicht unterscheiden lassen . . .». HEGETSCHWEILER

(1840) observes under *A. flexum*: »Zwiebel- oder capseltragende Dolde». In KUNTH's enumeratio (1843) *pulchellum* forms are found partly among the *pulchellum* species and partly in the 3 preceding species, *carinatum* L., *flavum* L. (*β tauricum*) and *nebrodense* GUSS. Under *A. carinatum* we find *β capsulifera*: »bulbillis in umbella laxa nullis. Varietas *β* omnino *A. pulchello* DON et *A. paniculato* REICHENB. Ic. 5 f. 604, quod vivum nondum examinare potui, simillimum est, tamen diversum videtur». REICHENBACH (1848) includes 3 species, all of which should be referred to *pulchellum* DON and are distinguished from one another by the shape of the perianth leaves, viz. 1060. *montanum* SIBTH. (perianthii partitionibus rotundato obtusis), 1061. *paniculatum* L. (perianthii partitionibus retusis) and 1062. *pallens* L. (peranthii partitionibus rotundatis, externis retusis). LEDEBOUR (1853) places types of *pulchellum* under *carinatum* (*β capsuliferum*) and *flavum* (*β pulchellum*): »variat floribus flavis v. luteis leviter purpurea suffusis v. sordide roseis».

In his monograph (1875), REGEL does not regard *A. pulchellum* as a species, placing it instead under the *flavum* species, where more or less red-flowered forms are found among the subspecies *β*, *γ*, *δ* and *ε*. He justifies his combining *pulchellum* and *flavum* in the following manner: »Unter *A. flavum* haben wir nach LEDEBOURS Vorgange, sowohl die gelbblumigen, als auch die rothblumigen mit langer Spatha, ausgebreiteter Dolde ohne Zwiebeln, hervorragenden Staubfäden und Blüthenstielen, die unter sich ungleich lang, vereinigt. . . Nun giebt es aber rein gelb blühende Formen, welche unsere Form *α* darstellen. Dann giebt es Formen mit gelben Blumen, die schon theils mit roth belegt sind, oder die mit grün und weiss nyancirt sind (*β*) und die allmälig zu den rosenroth und violett blühenden Formen übergehen». KOCH does much the same thing in his edition 1907, placing *pulchellum* as a subspecies of *flavum*. THOMÉ (1903) refers it to *carinatum*: »Zwei nur Kapseln tragende Abarten sind var. *capsuliferum* KOCH und var. *pulchellum* DON». ASCHERSON and GRÆBNER (1905) discuss the possibility of referring *pulchellum* to either *carinatum* or *flavum*: »Eine sehr kritische Pflanze, deren systematische Stellung einigermassen strittig ist. KOCH, HAUSMANN, KERNER u. a. haben sie mit *A. carinatum* verbunden, von der sie nur eine Form ohne Zwiebeln in Blüthenstande darstellen sollte. REGEL dagegen hat sie mit *A. flavum* vereinigt. Wir halten beides für wenig glücklich, zumal in einer so schwierigen Gruppe, wie sie die Gruppe *Macrospatha* darstellt. Wir halten *A. pulchellum* für eine Art der Gesammtart *A. paniculatum*, deren Arten alle zweifellos nahe mit einander verwandt sind». The species *pulchellum* is then divided by these authors into 2 subspecies. One of these subspecies, *ligusticum* A. GR., has a light-coloured perianth, and includes both REICHENBACH's *pallens* and REGEL's *β tauricum*. Two forms of this subspecies are distinguished, viz. the few-flowered *gracile* and the dwarfed *nanum*. The other subspecies is *valdensium* A. GR. with long, slender bulbs and graceful mode of growth. Its perianth leaves are broad and of a beautiful roseate colour.

The geographical distribution of *A. pulchellum* is the following: South France, the Pyrenees, Italy, the Balkan States, Asia minor, the Caucasus, Palestine, North Africa.

C. ALLIUM FLAVUM.

Purely yellow-coloured flowering forms of the *paniculatum* group were observed very early, probably owing to the strikingly beautiful flower-colour. Thus HALLER describes (1749) »Allium umbella non bulbifera lutea, vagina bicorni, foliis

teretibus». LINNÉ gives it as a species in his Species plant. (1753) and in Mantissa (1771). The same descriptions recur in GMELIN (1791) and WILLDENOW (1799). The difficulty of separating these yellow-flowered forms from more or less red-flowered forms soon begins to make itself felt in the literature. Thus MARSCHALL A BIEBERSTEIN (1808—1819) writes about *A. flavum*: »Apud nos semper corolla ex ochroleuco sordide purpurascente reperitur, quam varietatem et antiquiores jam commemorant, praeter colorem a varietate pure sulphurea, quam sistis JACQ. l. c. et Bot. Mag. 1330 nullatenus diversam». REICHENBACH (1848) reproduces (fig. 1063) a very many-flowered *flavum* type. REGEL's opinion of *flavum* has already been mentioned. Typical *flavum* corresponds to his subspecies α. BOISSIER (1884) includes a β *minus*, which is a dwarfed alpine form. The height, which is very variable in this species, is employed by ASCHERSON and GRÆBNER (1905) as the basis of the division of subspecies: *A.* Height 2—5 dm. To this class belong *typicum* and forms with greenish yellow perianth leaves, *tauricum* (= *ruthenicum* LANG). — *B.* Low forms with a height of up to 1,5 dm. Amongst these are found *Webbii* CLEM., which has »Stengel hin- und hergebogen», inflorescences few-flowered and flowers of a bright colour, and *Guiccardii* BOISS., which has the same general appearance but straw-coloured flowers. A small, erect, few-flowered type is *nebrodense* GUSS. These low *A. flavum* forms with a rather »distorted» manner of growth are very characteristic. I have several such forms in culture and their characteristic individuality has proved to be genetically fixed. They are no doubt alpine and subalpine ecotypes. It is this habitual character which causes me in the present paper to refer *flavum pumilum roseum* (v. p. 330) to *flavum*, in spite of the fact that its flowers are very nearly red. Its *flavum*-like manner of growth and appearance otherwise justify, in my opinion, this departure from consistency.

Between *pulchellum* and *flavum* there is a hybrid described »in Bulgarien gefunden, VELENOVSKY, Fl. Bulg. suppl. 1, 277 (1898)» (cited after ASCHERSON and GRÆBNER, 1905).

The geographical distribution of the species is: Provence, Dauphiné, Pannonia, Croatia, Dalmatia, Herzegovina, South Morovia, Siebenburgen, Galicia, South Russia, Caucasus, Western Asia.

D. ALLIUM CARINATUM.

The name *carinatum* originates from HALLER's (1749) description »foliis carinatis», but which referred to a type of *oleraceum*. LINNÉ's description of the species *carinatum* is found in Flora suecica (1745) and also in Species plantarum (1753): »Allium caule planifolio bulbifero, staminibus subulatis». In Mantissa (1771) the following addition is made, »spatha longissima, angustissima». WALDSTEIN and KITAIBL (1812) distinguish *flexum* from *carinatum*, the former being according to HEGETSCHWEILER (1840) the only typical *carinatum*, whereas *carinatum* L. is described in that work as »Bh. blass-rosenroth. Stgf. von der Länge derselben» and is consequently an *oleraceum*. This description is modified a little, however, in a note: »Auch No. 995 (*carinatum* L.) hat manchmal Staubgefässe, die doppelt so lang sind als die Bh., und ebenfalls zuweilen deutlich gekielte und zuweilen deutlich rinnige, etwas hohle, fast stielrunde Blätter». The difficulty of distinguishing *carinatum* from *oleraceum* is not eliminated until leaf-characters are entirely left out of account and classification is based entirely on the length of the filaments. This was done by KUNTH (1843) and *carinatum* becomes »staminibus edentulis, perigonio denique duplo

longioribus». REICHENBACH (1848) reproduces 3 species, which have bulbils and long stamens, but which are distinguished from one another by the nature of the perianth leaves: 1057. *carinatum* L. (perianthii partitionibus obovato-oblongis acutis), 1058. *violaceum* WILLD. (perianthio trigona truncato violacea apice albomarginato) and 1059. *asperum* DON. (perianthii partitionibus rotundato obtusis). GRENIER and GODRON (1855—56) include 2 species, *carinatum* L. and *flexifolium* JORD. The former has the subspecies *β consimile* JORD., which is characterized by pink flowers and only slightly projecting stamens. *A. flexifolium* is distinguished by its spherical, compact inflorescence and numerous bulbils.

REGEL (1875) divides *carinatum* into 3 subspecies: *α typicum*, *β violaceum*, both of which have predominantly glabrous leaves, the flowers of the former being light-coloured, those of the latter darker, and *γ asperum*, which is characterized by scabrous leaves. Scabrousness in the leaves is also employed by ASCHERSON and GRÆBNER (1905) as a basis of division, *β asperum* RGL. being separated from the other forms. The margin of its leaves and nerves are very scabrous, it is dwarfish, has narrow leaves, slender stems, small bulbils and small flowers and also narrow perianth leaves. The other forms of *carinatum* are divided by ASCHERSON and GRÆBNER according to the size of the perianth leaves: I. Perianth leaves 5—7 mm. in length. These are a. *flexum*, which is in turn divided into *denticulatum* A. GR. (stem low, bent, inflorescence many-flowered, beautiful purple-coloured perianth) and *consimile* GREN. and GODRON (stem vigorous, tall, bulbils pointed, perianth leaves pink, pointed, a form of perianth leaves which makes the stamens project only very little out of the flower). Further, b. *violaceum* RGL., which has dark lilac—purple flowers. II. Perianth leaves 2—5 mm. in length. Comprises a form with small flowers, *parviflorum* BECK. and a form with extremely small flowers, *montenegrinum* BECK.

The species has the following geographical distribution: South Sweden (Province of Skåne), Denmark (Funen, Bornholm), Germany (scattered localities in the north of Germany, e. g. Rügen), the British Isles, France, Spain, Italy, North Balkan, South and Central Russia.

E. ALLIUM OLERACEUM.

In his Allii libellus, HALLER (1749) reproduces 2 types of *oleraceum*: »26. Allium umbella bulbifera, vagina bicorni, foliis teretibus» and »27. Allium inodorum, umbella bulbifera, vagina bicorni, foliis carinatis». The latter is a tall form of *oleraceum* with light-coloured flowers. LINNÉ describes *oleraceum* (1745) thus: »Cepa capitulo bulboso prolifero, foliis semiteretibus novemangularibus scabris fistulosis» and (1753): »Allium caule teretifolio bulbifero, foliis scabris semiteretibus subtus sulcatis». GMELIN (1791) and WILLDENOW (1799) repeat this description. MERTENS and KOCH (1826) call attention to the variation in the flower-colour in *oleraceum*: »Die Farbe der Blüthe ändert ab, bald ist sie weisslich ins strohgelbe spielend und über den Rükken ihrer Blätter zieht ein grünlicher, oder bräunlicher, oder röthlicher Streifen, bald ist sie rosenroth mit gesättigtern Rückenstreifen». ELIAS FRIES (1828) distinguishes 2 subspecies on the basis of the types of leaves, viz. *β complanatum* (majus, foliis complanatis, floribus roseis) and *γ canaliculatum* with hollow leaves. These forms are still used in the literature. BLUFF and FINGERHUTH (1836) have *complanatum*, majus, and *canaliculatum*, maximum, and so has KUNTH (1843). As mentioned, KUNTH is the first to make the difference between *carinatum* and *oleraceum* entirely

due to the length of the filaments. In his description *oleraceum* is »staminibus edentulis, perigonium subaequantibus». *Complanatum* is classed as an own species by GRENIER and GODRON (1855—56) on the following grounds: »Il a les étamines de l'*A. oleraceum* et les feuilles de l'*A. carinatum*. La forme des feuilles ne permet pas de réunir cette plante à l'*A. oleraceum*, tel que LINNÉ l'a si bien décrit dans le Flora suecica». KOCH (1857) distinguishes between *α angustifolium* and *β latifolium*, a name changed to *complanatum* in a later edition (1892).

REGEL (1875) follows KUNTH in the limitation of *oleraceum* and *carinatum*: »Wir haben als Scheidungscharakter zwischen *A. oleraceum* und *A. carinatum* die Länge der Staubfäden angenommen, indem dies der einzige Charakter ist, durch den diese beiden Arten getrennt werden können». On the basis of features of the leaf and the colour of the flower REGEL separates the following subspecies: a. *typicum*, foliis teretiusculis, floribus virescentibus v. carneo-albidis v. lurido-rubescentibus viridique variegatis, b. *virens compressum*, c. *roseum teretifolium* and d. *roseum complanatum*.

A similar division is employed by ASCHERSON and GRÆBNER (1905). Thus the *complanatum* form is separated, and other forms with round leaves are divided into 2 groups: I. *angustifolium*, large and vigorous, and II. *alpicola*, a small form with scabrous leaves. *Angustifolium*, which is the commoner form, is again divided according to the following features: 1. the flower-colour, *virens*, *roseum*, *album*, 2. the nature of the leaf-margin, *leve*, *scabrum*, 3. the relative development of the flowers and bulbils, *pauciflorum*, *sterile* (no flowers at all). The form called *viviparum* has bulbils, developing on the mother plant. The species is treated in the same way by HEGI (1906) and ROUY (1910). The latter gives a good description of the *complanatum* race: »Tige relativement plus élevée (4—8 déc.); feuilles à peine fistuleuses, comprimées, presque planes, non canaliculées, plus larges (7—9 mill. de large au lieu de 3—5 mill.); ombelle multiflore à 30—50 fleurs, à bulbilles plus grosses, ovoides, obtuses ou apiculées».

The geographical distribution of the species: Scandinavia, Denmark, British Isles, Central Europe, North Spain, Italy, Upper Balkan, Central and South Russia, Siberia.

2. THE MATERIAL.

A short descriptive account will now be given of the forms in the *paniculatum* group investigated. These descriptions, along with the accompanying photographs, are intended to present a picture of the natural variation within the species. Since the various forms have been cultivated side by side in the same field for several years, most of the characters mentioned undoubtedly denote genetical differences. The numbers are my field numbers at Hilleshög. The place-names after my field numbers denote the origin of the form, the natural habitat, when given, otherwise an official botanical garden. The percentage of good pollen is given within brackets for most forms. The pollen analysis was performed in 1935 on aceto-carmine slides of ripe pollen grains. At least 200 pollen grains were counted from each form.

A. ALLIUM PANICULATUM.

141, 143. Munich (sub nomen *paradoxum*). Small, low form with almost white flowers (70 %, 95 %).

147. Parma (s. n. *baicalense*). My largest and most vigorous type of *paniculatum*. Stem more than $^1/_2$ cm. in diameter, not tall but very robust. Inflorescence many-flowered (150—250 flowers). Flower-colour golden brown. (95 %). (Fig. 1 *a*).

Fig. 1. *a*: *Allium paniculatum* 147, *b*: *A. pulchellum* 690.

180. Bucharest. Erect, slender plant, few-flowered inflorescences of a more »spongy» character. Flowers light pink, almost pure white. (92 %). (Fig. 2 *j*).

183. Kamianetz. Vigorous, erect, many-flowered type. Flowers light pink with darker lines along the middle of the perianth leaves. (95 %). (Fig. 2 *i*).

184. Cluj. Big and vigorous, very large, round bulbs (up to 2 cm.

in diameter), many-flowered, flowers quite brown. One of the parents of the synthetic *oleraceum*. (96 %). (Fig. 6 *a*).

227. Jassy. Vigorous and robust plant, many-flowered. Flower-colour whitish green with a brownish tint. (93 %). (Fig. 2 *h*).

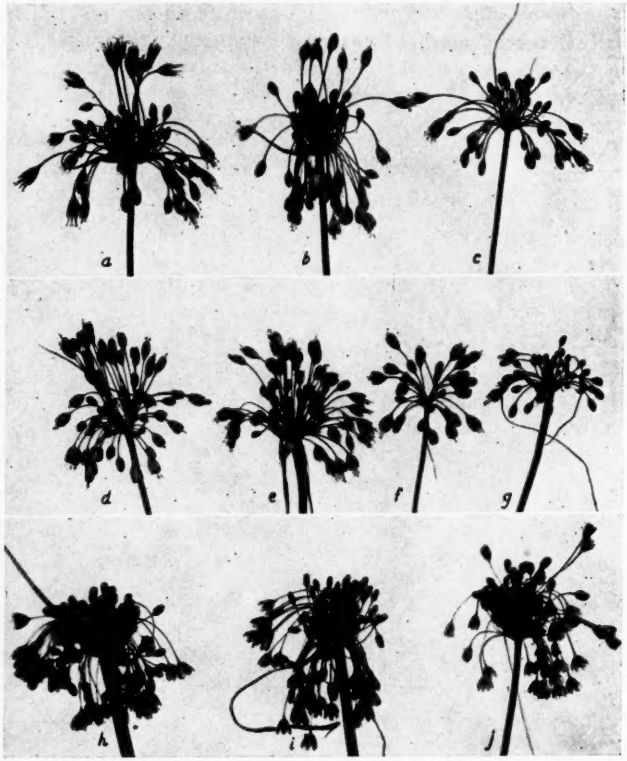

Fig. 2. *a—c*: *A. pulchellum*, *a*: 185, *b*: 182, *c*: 186; *d—g*: *A. flavum*, *d*: 195, *e*: 140, *f*: 856, *g*: 296; *h—j*: *A. paniculatum*, *h*: 227, *i*: 183, *j*: 180.

236. Pavia. Rigid, erect but not tall stems, many-flowered. Perianth leaves greenish with brown middle rib. (78 %).

259. Copenhagen. Rather slender, flower-colour very light lilac. (88 %).

674. Stockholm (s. n. *podolicum*). A low, slender, light green, densely tufted form, flower-colour light roseate. One of the parents of the synthetic *oleraceum*. (80 %). (Fig. 6 *b*).

B. ALLIUM PULCHELLUM.

139. Munich. A tall type with luxuriant many-flowered inflorescences. (72 %).

148. Helsingfors. Rather low, glaucescent, relatively few flowers per inflorescence (15—40), flower-colour greyish red. Pollen fertility low. (25 %).

179. Bucharest. Robust and vigorous, many-flowered, about 200 flowers per inflorescence. (78 %).

181. Gothenburg. Normal type. (88 %).

182. Tübingen. Normal type. (90 %). (Fig. 2 *b*).

185. Leyden. Normal type. (95 %). (Fig. 2 *a*).

186. Warsaw (s. n. *Porrum*). Only 20 cm. in height, relatively few-flowered. (92 %). (Fig. 2 *c*).

187. Cluj. Normal type. (95 %).

188. Cannes. Normal type. Flower-colour unusually light. Pollen of an abnormal shape, more rounded than normal, uneven size. (50 %).

196. Surrey (s. n. *carinatum*). Glaucescent. (94 %).

218. Wageningen. Low and slender. (36 %).

690. The firm of van Tubergen. Rather tall and vigorous type (30—40 cm. high), many-flowered. (70 %). (Fig. 1 *b*).

C. ALLIUM FLAVUM.

140. Toulouse (s. n. *insubricum*). Normal type. (84 %). (Fig. 2 *e*).

150. Erlangen. Very low form (15 cm. high), glaucescent. (85 %).

195. Geneva (s. n. *coeruleum*). Large, robust type, flowering rather late. (91 %). (Fig. 2 *d*).

222. Freiburg. Erect, slender, pure green non-glaucescent type. Small flowers on long, slender pedicels. This form flowers the latest of all my *flavum* types, in bud as late as 1st August. (95 %).

223. Vienna. Very small (10 cm. high) and few-flowered (20—30 flowers per inflorescence), pedicels strikingly short (1 cm. at most), which gives this form a peculiar appearance. (86 %).

296. Utrecht. Low, few-flowered, very glaucescent form. (Fig. 2 *g*).

297. Prague. Erect with rather short stem (30 cm. in height), vigorous and robust. Inflorescence large and luxuriant (up to 8 cm. in diameter), many-flowered (150—200 flowers per inflorescence). (85 %).

855. Munich. Normal type. (92 %).

856. Wageningen. Normal type. (95 %). (Fig. 2 *f*).

857. Sofia (s. n. *flavum* var. *minus*). A very small type with a

partially prostrate growth. It does not rise more than 10 cm. above the ground. Flowers early, small, few-flowered inflorescences. Pollen poor. (12 %).

D. ALLIUM FLAVUM PUMILUM ROSEUM.

Under this name an *Allium flavum* type (my Nos. 146, 767, 768) was sent me from the Botanical Gardens at Ottawa, which is noteworthy in many respects. In general appearance it resembles a low, spreading *flavum*, rather glaucescent. The growth is typical: the stems at first grow radially along the surface of the ground and then rise in the form of a curve. In this way the plant becomes very extended (Fig. 3 *a*).

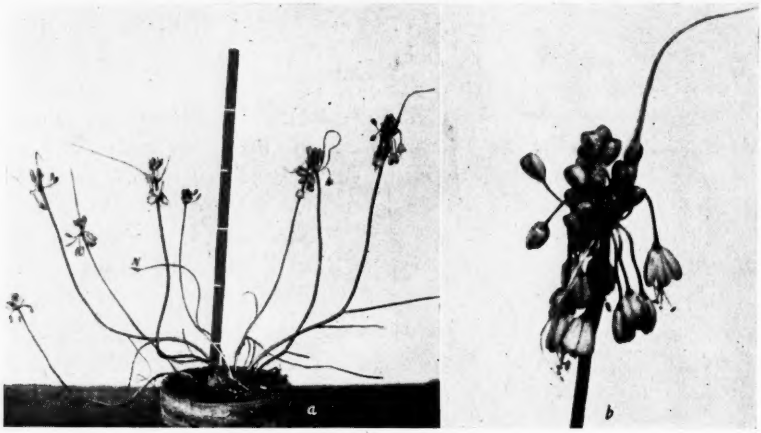

Fig. 3. *A. flavum pumilum roseum.*

Vegetatively it is exactly like *A. flavum*, but the colour of the flowers is never pure yellow but of a more or less dirty red with darker red streaks along the middle lines of the perianth leaves. The flowers are large, semi-spherical, and in the budding stage the perianth leaves are thick, almost fleshy. The inflorescences are few-flowered with 5—25 flowers in each. (Fig. 3 *b*).

The pollen is uneven, frequently to a great extent sterile. Pollen examination on different occasions have given varying percentages of good pollen: 22, 25, 40, 53, 88. As will be shown in a later chapter, meiosis in this form is of a characteristic type, differing from all other related diploid forms.

On account of its high degree of pollen sterility, among other

features, this form might be suspected of being a hybrid, for instance, between *pulchellum* and *flavum*. An argument against this view is the abundant seed-setting. One progeny after self-fertilisation (No. 1331, 12 plants) showed no segregation at all, but agreed entirely with the mother plant. As mentioned in the preceding section, red-flowered *flavum* types have also been described from nature. Perhaps they are segregation products of an old species crossing.

E. ALLIUM CARINATUM.

149. From the natural habitat at Tuna, east of Lund. A small, low, glaucescent type (20 cm. high). Flowers very dark red. Inflores-

Fig. 4. *A. carinatum*, *a*: 830, *b*: 149, *c*: 721.

cences relatively few-flowered (7—20 flowers). Bulbils non-viviparous, pollen fertility low. (20 %—30 %). (Fig. 4 *b*).

226. Lund, spontaneous locality in lawns in the Botanical Gardens. Low, few-flowered type. Similar to the preceding. (33 %).

938. From natural habitat, Helgonabacken, Lund. A taller plant than either of the preceding types, broad leaves, pure green in colour, evidently a shade-form. Bulbils somewhat viviparous on the mother plant. (30 %).

190. Erlangen. A low plant with broad leaves, flowering late. Few-flowered (0—5 flowers). Bulbils non-viviparous on the mother plant, small, pale and round. (20 %).

197. Geneva (s. n. *vineale*). Tall and uncommonly vigorous plant. Many-flowered (10—25 flowers), bulbils viviparous. (12 %).

198. Bucharest (s. n. *oleraceum*). 8—10 flowers per inflorescence. Bulbils viviparous. (12 %).

206. Rouen. 10—20 flowers per inflorescence. Bulbils partly viviparous. (20 %).

208, 224, 237, 242. Copenhagen. Normal type. 10—20 flowers per inflorescence. Flower-colour intensively red-lilac. Bulbils non-viviparous. (20, 26, 26 and 21 % respectively).

211. Bremen. Low and few-flowered (4—10 flowers). Bulbils characteristic, large, round, glossy white, falling off early. (18 %).

232. Groningen. 6—20 flowers per inflorescence. (23 %).

243. Paris. 7—15 flowers per inflorescence. (18 %).

244. Helsingfors. Normal *carinatum* type, 10—20 flowers per inflorescence. Flower-colour saturated red. Bulbils fall off shortly after they have begun to grow out. (34 %).

721. Berlin. Stout and rigid stem, fragile, few-flowered (5—15 flowers per inflorescence). Large flowers on short pedicels, often monstrosities. Bulbils very vigorously developed, viviparous. On the 30th July 1935 the bulbils on the mother plants showed shoots 12 cm. in length. (13 %). (Fig. 4 *c*).

830. Zagreb. Vigorous and tall type. Extremely many-flowered (50—70 flowers per inflorescence). Pedicels unusually long, flowers small. Bulbils not viviparous. A strikingly similar appearance to *A. pulchellum*. (25 %). (Fig. 4 *a*).

F. ALLIUM OLERACEUM.

136. From natural habitat at Örgryte, Gothenburg. Typical Swedish *oleraceum*; rather few-flowered, bulbils falling off. (85 %). (Fig. 5 *c*).

301. From natural habitat, Hildesborg, Landskrona. Resembles the preceding form.

1319. From natural habitat, Mentone, Kindly presented to me by the late Dr. TJEBBES. This form of *oleraceum* has quite a different appearance from the two preceding forms (Fig. 8 *b*). It is tall and vigorous, the stem is leafy high up. It is many-flowered (about 50 flowers per inflorescence). The bulbils are at least partly viviparous. This is a *complanatum* type (see p. 325).

144. Copenhagen. Few-flowered (0—4 flowers), perianth leaves

brown. The bulbils gradually become large, round and dark glossy. (48 %). (Fig. 5 *d*).

210. Saratov. Normal type. 10—25 flowers per inflorescence. Flowers unusually light in colour, brownish white. Pollen aborts early, shrunken, empty. (0 %).

212. Tabor. 15—20 flowers per inflorescence. Like form 1319; the bulbils are viviparous. (Fig. 5 *b*).

Fig. 5. *A. oleraceum*, *a*: 251, *b*: 212, *c*: 136, *d*: 144, *e*—*h*: 757.

219. Rouen. Normal type. (45 %).

247. Königsberg. Normal type. (88 %).

249. Liège. Normal type. (67 %).

251. Baarn. Very many-flowered, tall type (up to 60 flowers per inflorescence). (78 %). (Fig. 5 *a*).

752. Berlin-Dahlem. Tall, stout form, rather many-flowered (5—25 flowers). Perianth leaves deep brown. Bulbils often very large, spherical. (70 %).

757, 763, 777. Gothenburg. Rather frail and slender form. Monst-

rous. From the primary group of bulbils there arise small stems, which bear secondary bulbil groups. Flowers are generally absent altogether. Sometimes intermediate forms between flowers and bulbils are observed in the secondary bulbil groups. (Fig. 5 *e*—*h*).

3. EXPERIMENTAL WORK.

A. CROSSES IN DIPLOID SPECIES.

Crosses between the diploid species have been attempted in different directions but without any result. The cross *pulchellum* × *flavum* occasionally gave a few solitary seeds, which were, however, incapable of germination. On the other hand, *paniculatum*, crossed with other species, never showed any trace of seed-setting.

Fig. 6. *A. paniculatum*, the parent plants of synthetic *oleraceum*, *a*: the ♀ parent 184, *b*: the ♂ parent 674.

Crosses between extreme types within the species are easily successful. Of such crossings only one will be mentioned here, viz. the *paniculatum* cross which gave rise to the synthetic *oleraceum*. In the summer of 1934 *paniculatum* 184 ♀ was crossed with 674 ♂. Both these plants are reproduced in Fig. 6, from which it will be seen that they were very much unlike in appearance. One was a big, robust type, growing alone and having brown flowers, whereas the other was a slender, tufted type with light pink flowers. 40 flowers were crossed, 25 seeds were obtained, giving rise to 16 plants. All these plants were of the robust, solitary type, but among themselves they exhibited differences in several characters: height of plant, colour of flower, leafiness and so on (v. Fig. 7). 7 of them were as tall as or taller than any of the parents, the others being shorter. The colour of the flowers was chiefly brown but varied in darker or lighter shades. 15 of the plants,

however, were normal diploids, while one plant showed itself to be a tetraploid with the chromosome number 2n = 32. This plant (Figs. 7 *a* and 8 *a*) differed from the other plants by reason of its somewhat greater height, the colour of its flowers, which were greenish brown with darker brown lines along the middle ribs of the perianth leaves. But above all it differed from all sister plants in having bulbils in the inflorescences. This character, together with the doubled chromosome number, make it impossible, from a taxonomical point of view, to refer this form to *A. paniculatum* and it must be regarded as a form of *oleraceum*.

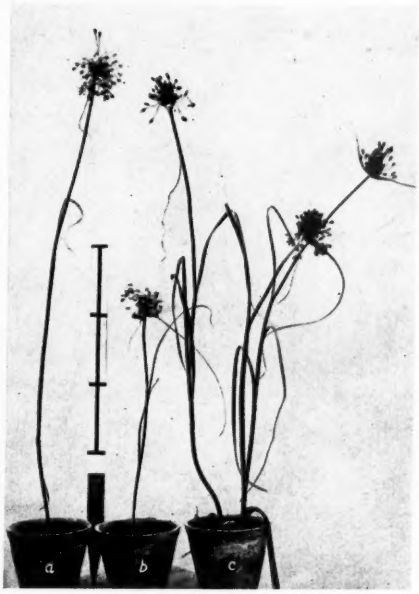

Fig. 7. *a*: The synthetic *oleraceum*, *b*—*c*: 2 of its sister plants.

As will appear from Fig. 7, this tetraploid plant nevertheless agreed with its sister plants to a great extent and differed quite considerably from our common northern types of *oleraceum*. In developing it at first proved to be very similar to *paniculatum*, indeed I did not discover the bulbils until the first flowers began to open, thus the bulbils were very small in size for quite a long time. The number of flowers in each inflorescence was much higher than is generally the case in the northern *oleraceum*, and the great number of small flower-buds entirely concealed the bulbils for a long time. Not one of my cultivated forms of *oleraceum* is exactly like this synthetic *oleraceum*, but, as I have already mentioned, I have in culture also very many-flowered forms of *oleraceum*, one of them in particular — No. 1319 from Mentone — is interesting in this connection. Fig. 8 shows the striking agreement existing between this Mentone form (on the right hand) and the new *oleraceum* type (on the left hand). Still, the new *oleraceum* plant had a greater number of flowers, in proportion to the bulbils, than the Mentone form (in the reproduced stem of the syn-

thetic *oleraceum* some 30 flower-buds have been removed from the front side in order to make the bulbils visible).

Another difference between the artificial *oleraceum* and our northern *oleraceum* form is the development and behaviour of the bulbils. In our wild forms the bulbils are generally oval and frequently glossy brown, and after flowering they usually fall off without germinating previously. In the new *oleraceum* the bulbils were pure green in colour

Fig. 8. *a*: The synthetic *oleraceum*, *b*: a spontaneous *oleraceum* type from Mentone, 1319.

and were so crowded that they were wedged into one another by means of even edges. Even before the flowers had faded away a green leaf began to develop in a number of the bulbils.

The pollen fertility of the new *oleraceum* was very good, there being about 90 per cent. good pollen, thus the upper limit as compared with that of spontaneous *oleraceum*. As is always the case, seed-setting in *oleraceum* was nil. While the diploid sister plants gave ample seeds the new *oleraceum* yielded only 6 seeds on the 3 existing scapes and these seeds were found to be empty.

How this chromosome doubling arose and where the first cell with a doubled chromosome number was situated is impossible to decide for certain. It is true that gigas pollen grains with doubled chromosome numbers frequently occur in *paniculatum*, as in nearly all species of *Allium*, and there is a possibility of course that such a pollen grain, which has been shown to be capable of functioning in other species, may have met with an unreduced female gamete. Still, the more simple explanation seems to me to be that this chromosome doubling is to be regarded as a somatic doubling which has occurred in one of the first divisions of the young embryo. In fact this is not the only example of such a phenomenon in *Allium*. In *A. Schoenoprasum* there is a parallel case, the description of which has not yet been published. This was also a case of 2 extremely dissimilar diploid forms, crossed with each other, and which already in F_1 gave a tetraploid gigas plant, alone amongst a number of normal sister plants. In a later chapter of this paper a similar case of chromosome doubling in F_1 between *A. pulchellum* and *carinatum* will be mentioned.

There is no dispute, however, as to the effect this chromosome doubling has brought about in *A. paniculatum*, that is, a transition from that species to *A. oleraceum*, a transition from one Linnean species to another.

B. SEED PROGENY OF ALLIUM CARINATUM.

A. carinatum, like *A. oleraceum*, is completely seed-sterile in nature. One of the main problems in my investigations of the connection between diploids and polyploids in the *paniculatum* group was to try to induce the triploid *A. carinatum* to produce seed. My attempts in this direction commenced in the autumn of 1930, when I thoroughly examined thousands of inflorescences of *A. carinatum* in natural habitats at Lund without being able to find a single seed. I then made attempts to stimulate the plant to set seed by removing the bulbils from the inflorescences at an early stage. I thought that in this way any embryos which might be formed would obtain a greater supply of nutritive material. I got this idea from WEBER (1929), who made use of it to induce *A. scorodoprasum* and *A. Moly bulbilliferum* to set seed. An attempt carried out on a small scale in 1931 failed however. In the following year the experiment was repeated on a larger scale, all the bulbils being very cautiously removed, while the flowers were still in a very early stage of budding, from a bulbil progeny of the Tuna form No. 149, consisting of about 300 plants. It was not enough, however,

to remove the bulbils only *once*, the operation had to be repeated about every other day during the entire period of flowering, for new bulbils, still larger and more vital than those removed, developed at once. In this way I really obtained the production of seed in some 30 plants, which resulted in 11 ripe, full seeds. These seeds, however, had much difficulty in germinating. They remained at first for a whole year between damp filter papers, and not until they had been exposed several times to cold shocks (up to 12 hours at a temperature of 5° C. below zero) was any germination obtained. At last 5 seeds germinated and gave rise to 5 plants. At first these plants were very frail, one or two

Fig. 9. 4 progeny plants from inbred *A. carinatum*, *a*: 2n = 17, *b*: 2n = 19, *c*: 2n = 30, *d*: 2n = 16.

of them remained for 4 months as germinal shoots only 1 cm. long, without any perceptible sign of growth. I nevertheless succeeded in keeping all the plants alive, and in the summer of 1934 the first flowers and bulbils were produced.

These plants, the inbred offspring of *A. carinatum*, were quite different in appearance, as was to be expected in a triploid progeny. 4 of them (Nos. 1—4) are shown in Fig. 9. Three of the plants, Nos. 2, 4 and 5, resembled the mother plant *A. carinatum*, having both flowers and bulbils. Two of the plants, Nos. 1 and 3, differed considerably, however, from wild *carinatum*. Thus No. 1 was entirely without bulbils (Fig. 10 *a*) and produced a much greater number of flowers than the mother plant. No. 3 again was entirely devoid of flowers, developing

instead at the tops collections of a few large bulbils, which developed viviparously on the plant. The height of the plants varied: the tallest was No. 2, reaching a height of 30 cm., Nos. 4 and 5 were a little shorter, about 25 cm. and the shortest was the bulbil plant No. 3, which did not grow to more than about 15 cm. high. These differences in height and characters otherwise proved to be constant from year to year and are now more conspicuous since the original plants have been divided vegetatively and have given rise to an entire plot of each form.

The chromosome numbers in the progeny were as follows: Two were pure diploids (Nos. 4 and 5), one was a trisomic (No. 1), one had 19 chromosomes (No. 3) and one was a hypotetraploid (No. 2) having 30 chromosomes. Thus in the majority of the progeny the chromosome number was lower than that of the mother plant. In this respect there is a distinct difference from the state of things in triploid progenies in other species of *Allium*, such as *A. Schoenoprasum* and *A. nutans*, where the chromosome number shows a tendency to increase from the diploid to the tetraploid stage. As far as *carinatum* is concerned nothing definite can be said on the evidence of these plants only, but the tendency here is at any rate quite another.

The chromosome number $2n = 19$, shown by the *carinatum* offspring No. 3, belongs to those chromosome numbers which are most difficult of realization in *Allium*. The chromosomal unbalance is here evidently great. Thus in a triploid progeny of *A. Schoenoprasum* I found the number 19 only 4 times amongst 619 plants examined (LEVAN, 1936 a). The *A. carinatum* plant containing 19 chromosomes indicates also by its monstrous appearance that the chromosomal balance is seriously disturbed, even if in this case the viability of the plant does not seem to be greatly reduced.

Of great interest from the point of view of species formation is the trisomic plant, No. 1, which is entirely without bulbils (Fig. 10 *a*). Morphologically it has changed from *A. carinatum* to *A. pulchellum*, which differs from *A. carinatum* only in being devoid of bulbils. It is true that this type is trisomic and consequently not stabilized, and thus its agreement with *pulchellum* is only apparent. The pollen fertility in this form is low (23 %) and the small number of seeds obtained by isolation were incapable of germinating.

Both the 16-chromosome forms, Nos. 4 and 5, were rather similar to each other and, as already mentioned, both of them were typical *carinatum*. Their pollen fertility was very good (95 % and 78 % respectively). In 1935, 14 seeds were obtained after isolation from the

carinatum plant No. 4, 2 of which germinated, 11 seeds were obtained from plant No. 5, 1 of which germinated. One of these 3 plants, which was thus a I_2 of wild *A. carinatum*, was of interest because it had no bulbils and was in other respects exactly like *A. pulchellum*. It had 16 chromosomes and its pollen fertility was good (92 %) but it was less seed-fertile than spontaneous *A. pulchellum*, only 5 seeds being obtained after free-flowering this year.

Fig. 10. *a*: *carinatum* 1 ($2n = 17$), the plant is entirely devoid of bulbils, *b*: *carinatum* 5* ($2n = 16$), normal appearance of *carinatum*.

The 30-chromosome plant, which was also a typical *A. carinatum*, externally exhibited the best vitality of the five plants. The pollen fertility, however, was much lower than in the diploid plants (38 %). No seed-setting has been obtained after this plant.

These inbreeding experiments with spontaneous *A. carinatum* have thus resulted in the production of different types containing 16 chromosomes, among them being a I_2 plant which cytologically as well as morphologically forms a transgression to the species *A. pulchellum*. As will be shown later on in the cytological section, the difference between the *carinatum* and the *pulchellum* genomes is not great.

C. CROSSES BETWEEN ALLIUM CARINATUM AND OTHER SPECIES.

a. *Crosses with A. pulchellum.* — Crosses between natural triploid *carinatum* and *pulchellum* have been attempted, although on a small scale, but no results have been obtained. On the other hand, crosses between diploid *carinatum* and *pulchellum* succeeded very easily, quite as easily as variety crosses in *pulchellum.*

Thus in the summer of 1934 three inflorescences of the two *pulchellum* forms, Nos. 182 and 185, were crossed with pollen of *carinatum* No. 4. The former cross gave 70 ripe seeds, the latter yielded about 150. A large percentage of these seeds germinated and gave rise to Nos. 1325 and 1326, which after wintering in the field flowered for the first time this year, the number of plants then being 45 and 110 respectively. As mentioned, the mother plants were typical *pulchellum* and *carinatum* No. 4 was a typical 16-chromosome *carinatum* plant furnished with bulbils, which when inbred gave one plant with bulbils and one plant without bulbils.

Nos. 1325 and 1326 showed a segregation of the characters presence—absence of bulbils. The following numbers of plants were found:

No.	With bulbils	Without bulbils	Total
1325	15	28	43
1326	51	58	109
Total	66	86	152

The presence of bulbils seems to be dominant and the father plant must have been heterozygous for this character. It is, however, uncertain whether there is a simple 1 : 1 segregation, for it was by no means easy to classify the absence of bulbils as it might happen that at the close of the period of vegetation bulbils developed on plants that had previously been without bulbils. The above classification was made at the end of August, an estimation made 3 weeks earlier gave a much greater percentage of bulbil-free plants. On the whole this character manifested itself in a rather fluctuating manner in these two numbers. Certain plants had such extremely small bulbils concealed in a wealth of flower-buds that only a careful examination could disclose them, while other plants had only one or two flowers and many large prominent bulbils, and between these two extremes there were all sorts of transitional forms. The variation was of a similar nature to that

occurring in spontaneous *carinatum*. In the numbers there thus occurred pure *pulchellum* types as well as a pattern-card of all possible kinds of *carinatum* forms.

The chromosome numbers in root-tips were determined for 35 plants in No. 1325 and for 37 plants in No. 1326. As was to be expected, the chromosome number in these plants was 16. There were 3 exceptions from this rule, however, viz. 1325—3 and 17, both of which had $2n = 17$, and 1326—20, which was a tetraploid with $2n = 32$. These three divergent plants, along with a typical diploid plant of the bulbil-free type, are shown in Fig. 11. The 3 divergent plants were all much below medium height, and the two trisomes (Fig. 11 *b*, *c*) were shorter than the tetraploid (Fig. 11 *d*). The low growth of the tetraploid as compared with the diploid need not be interpreted as the absence of the gigas type, but is to be regarded simply as being due to the more retarded rate of growth that tetraploids frequently exhibit as compared with diploids (as for instance in *A. Schoenoprasum*). The tetraploid had the normal appearance of *carinatum*, thus bearing bulbils. This was also the case with one of the trisomes, viz. 1325—3, while the other trisome, 1325—17, was without bulbils.

Fig. 11. Instances of F_1 plants between *pulchellum* and *carinatum* 4, *a*: $2n = 16$, *b*—*c*: $2n = 17$, *d*: $2n = 32$.

These aberrant plants arose, as far as the trisomes are concerned, owing to the formation of solitary 9-chromosome sex cells, a phenomenon that has been observed occasionally, although very rarely, in the pollen of diploid species. As regards the tetraploid, the origin here, as in the new type of *oleraceum*, is in my opinion due probably to a somatic chromosome doubling. In the same cross there appeared a plant, No. 1326—77, in which 2 of the roots investigated had 32 chromosomes

and another root had 16. In the flowers, however, all the somatic tissue had 16 chromosomes. Here the chromosome doubling had occurred later during the ontogenetic development and given rise to only certain sectors with the higher chromosome number.

b. *Crosses with A. flavum.* — Crosses between common *A. flavum* and *A. carinatum* have failed so far. But a cross made in 1934 between *A. flavum pumilum roseum* 767 ♀ and *carinatum* 4 ♂ gave rise to 7 seeds, one of which germinated. The plant was certainly very frail but I succeeded in keeping it alive over the winter and getting it to flower this summer. Fig. 12 shows the appearance of the plant at the beginning of the flowering stage. In habit the plant agrees mostly with *carinatum*. The typical arched growth of *flavum pumilum roseum* (Fig. 3) is not seen in the hybrid, the stems growing straight up as in *carinatum* (Fig. 12). The plant had bulbils, but as shown in the figure they were very small and poor. The colour and the shape of the flowers agree entirely with *flavum pumilum roseum*. The chromosome number was 16. In the cytological section it will be shown that the type of meiosis in the hybrid is more in agreement with the mother plant than with *carinatum*. Pollen fertility was poor, and I did not succeed in obtaining any seed progeny this year.

Fig. 12. F_1 between *A. flavum pumilum roseum* and *carinatum* 4.

4. SUMMARY OF THE MORPHOLOGICAL SECTION.

Before proceeding to give a description of the cytology of the *paniculatum* group it would perhaps be appropriate to recapitulate here the gist of the preceding chapters. It is evident that the *paniculatum* group constitutes a variational sphere which is clearly differentiated from other species of *Allium*, within which sphere all forms are very closely related. The group contains an enormous number of forms, but this does not prevent the group from being fairly easily divided into 5 species. The species *pulchellum* has, however, caused systematists a certain amount of trouble, it has sometimes been made into a species

of its own, sometimes referred to *carinatum* and sometimes to *flavum*. The results of my experiments make these conditions comprehensible, the species *pulchellum*, *flavum* and *carinatum* having shown themselves to be built up of practically the same genome. That is also the case with regard to *paniculatum* and *oleraceum*, between which systematists have occasionally, although more seldom, hesitated to draw a boundary-line.

Judging from the results of my investigations it is in my opinion of minor importance whether we undertake any alterations in the taxonomy of the *paniculatum* species or not. The most difficult species in nature to separate are *flavum* and *pulchellum*, between which there are transitional forms. Thus my *flavum pumilum roseum*, for instance, was habitually a typical *flavum* form, but the colour of its flowers was more or less reddish. These intermediate forms are no doubt exceptions and hardly justify a combining of *pulchellum* and *flavum*. If one wished to combine the species within the *paniculatum* group thoroughly, the resulting large species would *not* be ASCHERSON and GRÆBNER's collective species (*paniculatum*, consisting of *paniculatum*, *flavum*, *pulchellum* and *oleraceum*, comprising *oleraceum* and *carinatum*) but would naturally be 1. *paniculatum* with the tetraploid race *oleraceum*, and 2. *pulchellum—flavum* with the triploid race *carinatum*. But as it is in general practice very easy to separate the 5 *paniculatum* species there does not seem to me to be any reason why such a fusion should be made. There is still less advantage in dividing these 5 species into a number of minor species, as some investigators have attempted to do. These minor species are, it is true, for the most part constant and correspond to ecotypes. The more characteristic of them, from a morphological or phytogeographical point of view, no doubt deserve the designation of subspecies.

II. CYTOLOGICAL SECTION.

1. TECHNIQUE.

In studying the root-tip chromosomes and the chromosomes in the first division of the pollen grain the same method was employed as that described by me earlier (LEVAN, 1932), viz. root-tip chromosomes were fixed in NAVASHIN's fluid and slides of pollen fixed in BOUIN-ALLEN's fixative. On the other hand, in this investigation the slides of the meiosis in the pollen mother cells were produced by a method which I have not previously used for *Allium*. As this method gave much better results than ordinary NAVASHIN fixation, which I had previously used

almost exclusively for meiosis of *Allium*, a short description of the method should be given here.

Slides of pollen mother cells were fixed in the osmic acid fixation, recommended by GEITLER (1934, p. 287), in the proportions 20 cc. of 1 % chromic acid solution, 5 cc. of 2 % osmic acid solution, 3 drops of glacial acetic acid. The best way to carry out the fixation is to pour a few drops of the fixative over the slide with the smeared pollen mother cells. By this means only a very small quantity of fixative is used for each slide. After a fixation period of only 20—30 seconds the fixative is washed off in running distilled water, which is allowed to run over the slide for 30 seconds. While there is still a sufficient quantity of water covering the slide a few drops of a 2 % hydrogen peroxide solution are added by means of a glass-rod. The bleaching is controlled under the microscope. When the pollen mother cells are absolutely white and clear, the slide is again rinsed in distilled water for a few seconds. The slide is then placed in a 1 % gentian violet solution for 30 seconds—1 minute. If allowed to remain longer than this time in the stain the slide will be useless, as the plasm will retain the colour very stubbornly. After staining the slide is treated in the usual manner. In spite of the short time of staining it may happen that the chromosomes are so deeply coloured that in addition to the destaining in oil of cloves the slide must be dipped into acid alcohol (100 cc. of 95 % alcohol + 1 drop of concentrated HCl). The slide is then embedded in condensed cedar oil.

This method is exceedingly suitable for all species belonging to the *paniculatum* group, and makes it possible to analyse the chromosomes of entire pollen mother cells, even in polyploid forms. The method can also be used for other species of *Allium*, but not all, and frequently only after some modification of the composition of the fixative. Whereas species such as *A. Cepa*, *fistulosum*, *Porrum*, *nutans* gave beautiful slides by this method, it was useless, for instance, for various forms of *A. Schoenoprasum*.

Meiotic prostages before diakinesis were poorly fixed by this method. Another disadvantage of the method is that the slide does not keep so well as those prepared and stained in the ordinary manner.

All pictures in this paper are drawn with the aid of the following system of lenses: Zeiss apochromatic immersion objective 120, compensating ocular 20, which gives a magnification of about 4300 times. In the reproduction the pictures have been reduced to $^2/_3$ of their original size.

2. SOMATIC CHROMOSOMES.

All the forms in the *paniculatum* group investigated have a similar chromosome form. In fact only one form of chromosome occurs in the entire group, the two-armed type with arms of approximately equal length. Terminally or subterminally inserted chromosomes, of which at least one occurs in every chromosome set in the majority of the *Allium* species, are entirely absent in the *paniculatum* group. Within the idiogram rather slight differences in size can be seen between the chromosomes. At any rate it is not possible to identify any special chromosome in any species. I have occasionally noticed slight differences in chromosome size between the species, but I consider them as significant only in one case. This refers to *A. flavum pumilum roseum*, which differs from all other forms in having considerably longer chromosomes and a much greater chromatin mass in both mitosis and meiosis. While the longest chromosome in the first pollen division of other types never exceeds 8 μ, that in *A. flavum pumilum roseum* amounts to 11 μ in length.

Figs. 13 *a—f* show the first pollen division in some of the forms examined. Fig. 13 *f* is a 15-chromosome plate from the synthetic *oleraceum*. Its chromosomes have identically the same appearance as the chromosomes of spontaneous *A. oleraceum*, drawings and microphotographs of which have already been published (v. LEVAN, 1933, Figs. 1 *c*, *d*. and 4 *d*).

Most of the forms of the *paniculatum* group which I have investigated are entirely without satellites. There are, however, exceptions to this rule. I have now and again observed satellites in *A. flavum* and in one *carinatum* plant (No. 232 from Groningen) there appeared rather regularly in root-tips 1 satellite chromosome per plate. But in the forms used in the crosses, which I have studied very closely, no satellite was ever observed. A very astonishing feature therefore was the regular occurrence of 2 chromosomes with large satellites in several of the examined plants of Nos. 1325 and 1326 (F_1 between *pulchellum* and *carinatum*). Figs. 13 *g—l* show a somatic plate from such a plant, along with some instances of satellite chromosomes. Pollen divisions and root divisions of both the parent plants were examined but no satellite was ever detected. In crosses, however, at least 12, probably more, of the plants investigated had such satellite chromosomes.

This is evidently a case of what may be called »negative amphiplasty», the hybrid plants showing satellites while the parent plants are

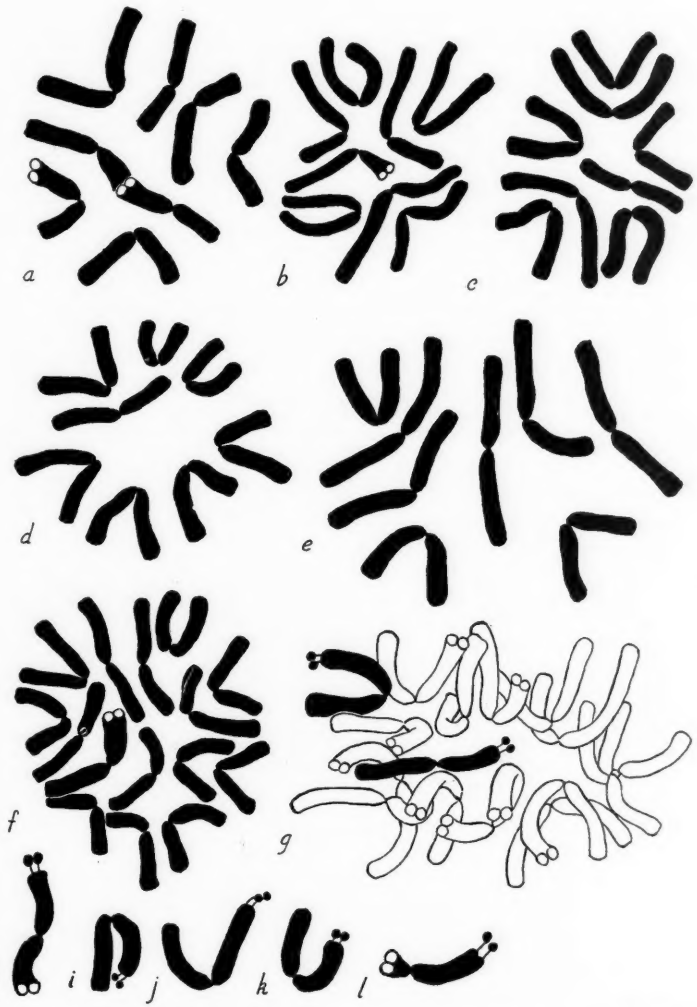

Fig. 13. *a—f*: First pollen division of *a*: *A. pulchellum* 179, *b*: *paniculatum* 1094, *c*: *carinatum* 4, *d*: *flavum* 856, *e*: *flavum pumilum roseum*, *f*: synthetic *oleraceum*; *g*: root cell mitosis of *pulchellum* × *carinatum*, *h—l*: instances of satellite chromosomes in this hybrid. — × 2900.

without satellites. The formation of the satellites must be due to the new environment which the chromosomes from the two species create

when they are brought together in the plasm of one of the species. This appears to me to be compatible only with the assumption that the satellites are manifestations of a genetic reaction and have nothing to do with simple structural changes. Whether this reaction brings about a complete organisation de novo of the satellites or whether it only produces changes in the contraction in the satellite chromosomes, whereby a previously invisible constriction comes to light, is impossible to judge from these cases. As far as Nos. 1325 and 1326 are concerned the latter alternative appears to be the more probable for the following reason. In those plants in which the satellite chromosomes are quite visible at metaphase it appears, as has already been demonstrated in several plants, among them *Allium*, that during the prophases the satellite chromosomes are associated with the nucleolus. It now appears that even in plants in which no trace of any satellite is visible during metaphase nevertheless in the first pollen mitosis one chromosome is associated with the nucleolus during prophase. In the tetraploid plant 1326—20, which in spite of ideal fixation did not exhibit any satellite at metaphase, there occurred at prophase in the pollen 2 nucleoli, which were associated each with one chromosome. In the prophases of such plants, which have no satellites at metaphase, it is sometimes possible with the aid of the nucleolus to identify the satellite at one end of the chromosome united to the nucleolus. These facts indicate that the absence of the satellites during metaphase is due only to their fusion to the chromosomes. This agrees with NAVASHIN's observations in *Crepis*. In hybrids with amphiplasty an increase in the volume of the satellite-bearing arm was occasionally observed at the same time as the disappearance of the satellite (NAVASHIN, 1934).

3. MEIOSIS.

A. ALLIUM PANICULATUM, PULCHELLUM AND FLAVUM.

Chromosome pairing at meiosis in diploid *Allium* species has been closely described several times. The species dealt with in this investigation show normal behaviour during meiosis. The pairing of chromosomes is good, 8 bivalents being formed and univalents being exceptions. The bivalents are usually ring-shaped. The absence of subterminally inserted chromosomes predispose to this bivalent form. Figs. 14 *a*—*c* show analysed metaphase plates of the diploid species. Special note should be made of Fig. 14 *a*, which shows no less than 5 rod-shaped bivalents, an exceptional condition in normal *paniculatum*. The plate reproduced indeed originates from a hybrid between two ex-

treme types of *paniculatum*, in fact from plant No. 1094—3, a sister plant of the synthetic *oleraceum*. As shown by Table 1, this variety hybrid exhibited a much smaller number of chiasmata per cell than

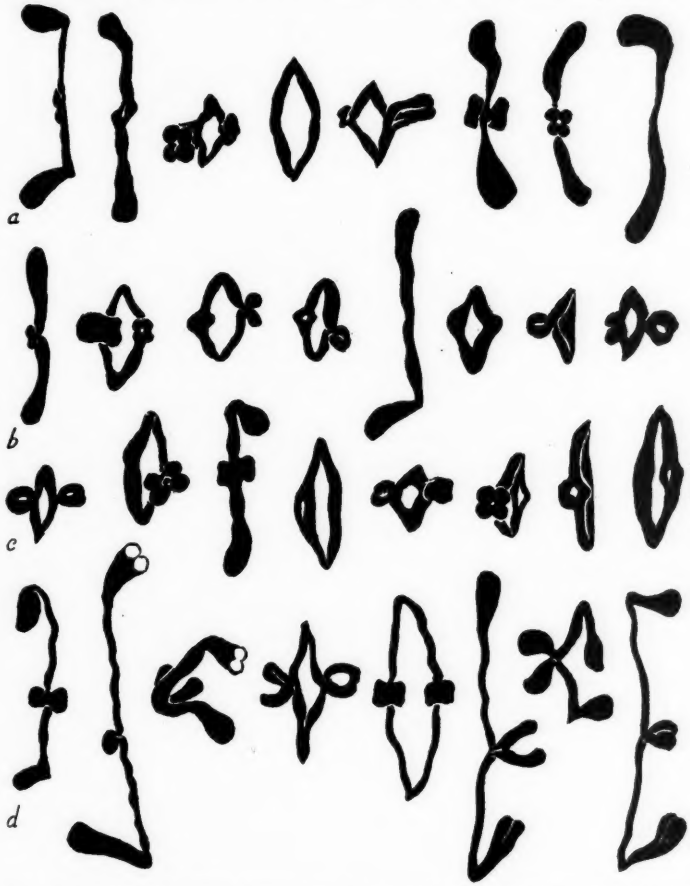

Fig. 14. Meiosis in diploid forms, *a*: *A. paniculatum* 1094, *b*: *pulchellum* 179, *c*: *flavum* 856, *d*: *flavum pumilum roseum*. — × 2900.

normal *paniculatum*, viz. 13,7 chiasmata per cell as compared with 18,7 in *paniculatum* 259.

Otherwise the chiasma frequencies of the diploid species were similar: an average of 16—18 chiasmata per cell, 3—5 of which were terminalized at metaphase. In sharp contrast to these species is that

TABLE 1. *The number of chiasmata in diploid forms.*

Form	Field number	xx/II					xx/cell														Mxx/ chro- mo- some	Mxx/cell		Term. coeff.
		0	1	2	3	4	7	8	9	10	11	12	13	14	15	16	17	18	19	20		Total	Term.	
paniculatum	259	—	1	57	16	6	—	—	—	—	—	—	—	—	—	—	1	3	4	2	1,17	18,7 ± 0,29	3,3	0,176
	1094—3	2	23	51	4	—	—	—	—	—	1	—	4	2	2	1	—	—	—	—	0,85	13,7 ± 0,43	4,7	0,343
pulchellum	477	—	15	52	12	1	—	—	—	—	—	—	—	1	3	3	2	1	—	—	0,99	15,9 ± 0,36	3,3	0,208
	179	—	13	58	7	2	—	—	—	—	—	—	2	1	5	1	1	—	—		0,99	15,8 ± 0,37	3,4	0,215
flavum	856	1	5	56	14	4	—	—	—	—	—	—	—	—	3	2	3	1	1		1,09	17,5 ± 0,41	4,6	0,263
flavum pumilum roseum	767	4	52	21	3	—	1	—	2	4	1	1	—	1	—	—	—	—	—	—	0,64	10,2 ± 0,55	1,2	0,122
carinatum	4	—	14	50	13	3	—	—	—	—	—	—	—	3	—	6	1	—	—		1,00	16,5 ± 0,32	2,2	0,133
	5	2	17	44	15	2	—	—	—	—	—	2	1	2	1	1	2	1	—		0,99	15,8 ± 0,65	2,3	0,146
pulchellum × *carinatum*	1325—1	1	24	53	2	—	—	—	—	—	1	—	3	4	2	—	—	—	—	—	0,85	13,6 ± 0,35	2,4	0,176
	—14	7	21	48	3	1	—	—	—	1	1	3	—	3	1	1	—	—	—	—	0,82	13,0 ± 0,57	3,1	0,238
	—16	—	21	67	2	—	—	—	—	—	—	—	3	3	4	—	—	—	—	—	0,88	14,1 ± 0,26	1,4	0,099
	1326—19	2	28	49	1	—	—	—	—	—	1	3	3	2	1	—	—	—	—	—	0,81	12,9 ± 0,36	2,8	0,217
	—27	5	20	54	1	—	—	—	—	—	1	4	1	2	1	1	—	—	—	—	0,82	13,1 ± 0,48	2,9	0,221
	—40	1	45	33	1	—	—	—	—	3	2	3	2	—	—	—	—	—	—	—	0,72	11,4 ± 0,37	1,8	0,158
	—75	1	10	64	5	—	—	—	—	—	—	—	1	—	4	5	—	—	—	—	0,96	15,3 ± 0,29	3,4	0,222
	—96	1	8	63	8	—	—	—	—	—	—	1	—	1	1	3	3	1	—	—	0,99	15,8 ± 0,53	3,4	0,215
fl. p. r. × *car.*	1327—1	11	50	19	—	—	1	3	[illegible]	2	1	—	—	—	—	—	—	—	—	—	0,56	8,9 ± 0,36	0,8	0,090

form of *A. flavum* which has been named *pumilum roseum*. Its behaviour during meiosis is very characteristic (Fig. 14 *d*). In the first place the very shape of the bivalents is different, the chromosome segments situated between the pole-thread insertions and the existing chiasmata are always unusually prolongated, as they would be if the localized force was powerful and the resistance of the chiasmata to terminalisation was strong. This is a characteristic feature of the form in question and is not due to chance occurrences such as fixation, weather etc. I have observed meiosis in this form on several occasions during the last 3 years and have always been struck by the difference in its meiotic morphology from that of other closely related forms. In addition to its special bivalent form, *flavum pumilum roseum* is also characterized by having a much smaller number of chiasmata than the preceding species, in 10 cells the number varied between 7 and 14, the average being 10,2. Univalents are common and rod-shaped chromosomes with only one chiasma constitute the dominating form of bivalent. Another characteristic feature of the meiosis of this form is the low degree of terminalisation. This depends however on the high frequency of rod-shaped bivalents, in which terminalisation is always lower than in the rings. If the degree of terminalisation of the existing rings in *flavum pumilum roseum* is compared with the corresponding figures in *flavum* no noteworthy difference will be found.

B. SYNTHETIC OLERACEUM.

Meiosis in the synthetic *oleraceum* coincides with that in spontaneous *oleraceum*, as already described (LEVAN, 1933). The most characteristic feature is the formation of trivalents and quadrivalents. The quadrivalent frequency per cell varies between 0 and 6, the usual number being 2—3. Trivalents are always accompanied by an equal number of univalents. In the cell analysed in Fig. 15 *a* the chromosomes were present in the following configurations: 3 IV, 1 III, 8 II and 1 I.

The quadrivalent form is the same as that in spontaneous *oleraceum*: chains and simple rings being most common, but all possible types of quadrivalents have been observed with the exception of the quadruple arc. Fig. 15 *b* shows a number of quadrivalents, chosen at random. The last quadrivalent in the second row, four rods joined at one point, has not been observed before in *A. oleraceum*, but certainly in other species of *Allium*, e. g. *Schoenoprasum*.

The number of chiasmata per cell is approximately 30. Entire cells

were, however, difficult to analyse in this form. Nevertheless, in order to obtain an average value of the chiasma frequency, I recorded the

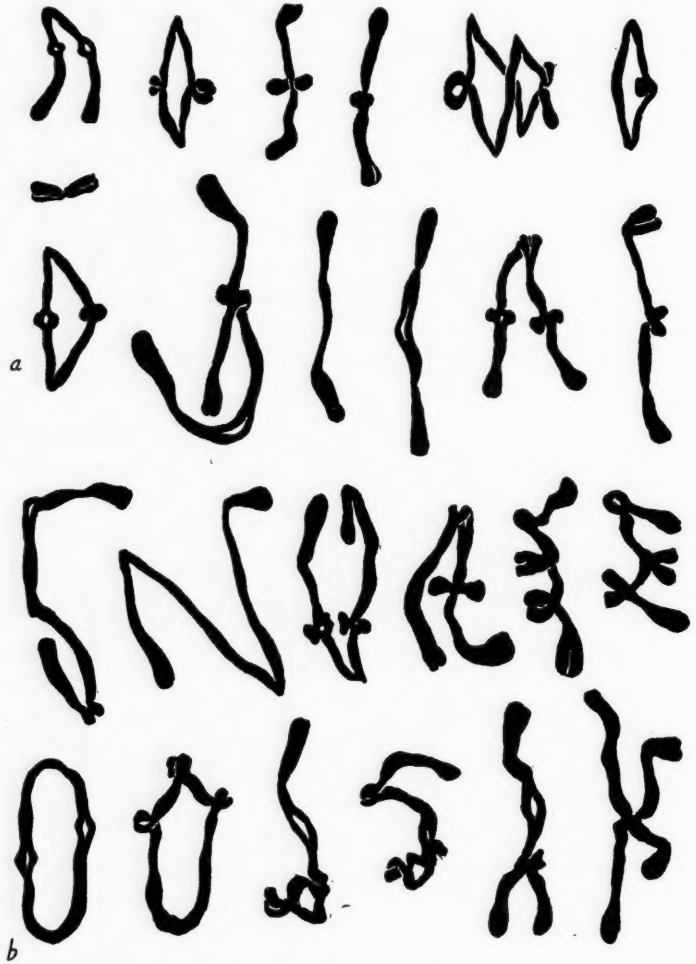

Fig. 15. Meiosis in synthetic *A. oleraceum*, *a*: one metaphase plate drawn separately, *b*: instances of quadrivalents. — × 2900.

number of chiasmata in 100 bivalents chosen at random. The following were the chiasma frequencies found:

Number of xx:	0	1	2	3	4	Total xx	xx/II	Term. xx/II	Term. coeff.
Number of cases	5	22	52	17	4	193	1,93	0,49	0,254

The calculated number of chiasmata per cell will thus be 31,3, a figure which is probably too low. Bivalents with only 1 chiasma can be easily seen because their arms project outside the equatorial plate, whereas bivalents with 2 or more chiasmata are more difficult to detect. The estimation indicates quite plainly, however, a higher value for the number of chiasmata per chromosome than that shown by the diploid sister plants, and even a somewhat higher value than that normally possessed by the *paniculatum* species.

C. SPONTANEOUS ALLIUM CARINATUM.

Fig. 16 *a* shows the meiotic chromosomes of the spontaneous triploid species *A. carinatum*. A high degree of trivalent formation is present, from 4 to 8 trivalents per cell being formed. The most common form of trivalent in this species is a chain of 3 chromosomes, the middle chromosome being bent in such a manner that the entire trivalent has the appearance of the letter V. In 10 analysed cells the following chromosome configurations were recorded:

Number of cases	Configuration
1	8 III
3	7 III, 1 II, 1 I
1	7 III, 3 I
4	6 III, 2 II, 2 I
1	4 III, 4 II, 4 I

The number of chiasmata per cell in the 10 analysed cells was:

Number of xx:	18	19	20	21	22	23	M xx/cell	M term. xx/cell	Term. coeff.
Number of cases:.....	2	2	1	1	1	2	20,2	5,4	0,260

Thus there was an average of 0,85 chiasmata per chromosome. The terminalisation is about twice as great as that in diploid forms of *carinatum*.

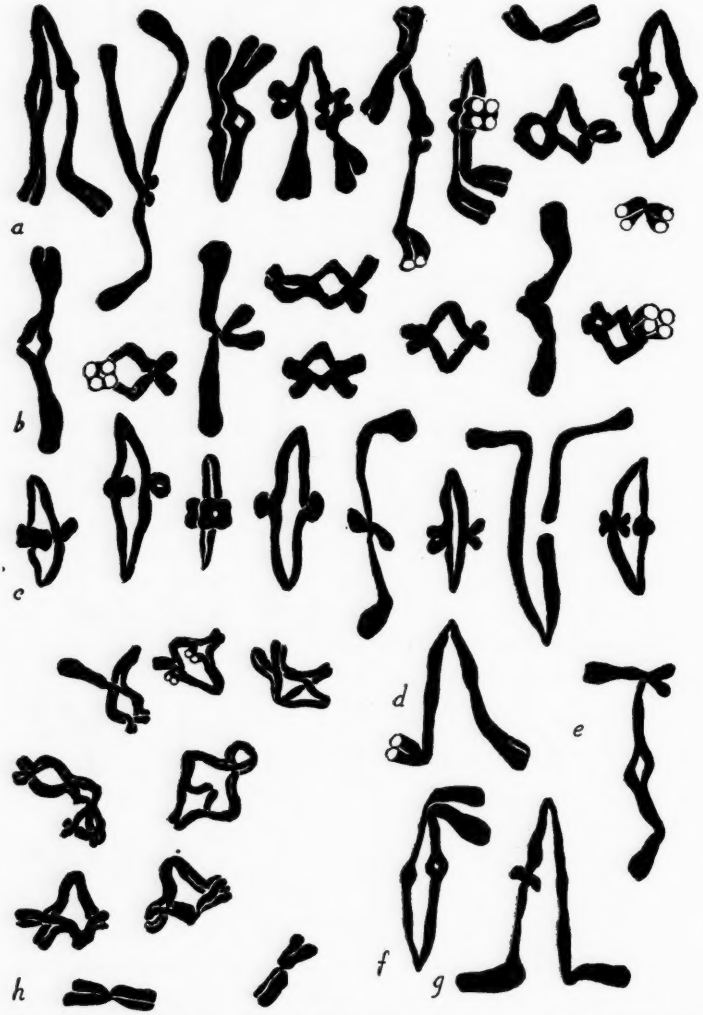

Fig. 16. Meiosis in different forms of *A. carinatum*, *a*: the spontaneous triploid species, *b*: *carinatum* 4 ($2n = 16$), *c*: *carinatum* 1 ($2n = 17$), *d—g*: trivalents from this plant, *h*: *carinatum* 5 ($2n = 16$), metaphase I in polar view. — × 2900.

D. INBRED PROGENY OF SPONTANEOUS ALLIUM CARINATUM.

a. *The diploids, carinatum 4 and 5.* — These have on the whole an identical meiosis. Chromosome pairing is quite as good as in the

spontaneous diploid species (Table 1). 8 bivalents are formed, which most frequently assume the shape of rings but occasionally 1—3 of them will be rod-shaped (Fig. 16 *b*).

A dissimilarity was observed, however, between the two forms 4 and 5. This difference is possibly due to only a chance occurrence, in *carinatum* 5 the pollen mother cells were only fixed on one occasion (3 slides). As shown in Table 1, *carinatum* 5 sometimes contained univalents, but no univalents were ever discovered in No. 4. Of 50 examined cells of *carinatum* 5 10 had 2 univalents. A cell of this kind is reproduced in Fig. 16 *h*. Although everything points to the fact that spontaneous *A. carinatum* consists of 3 structurally similar genomes, there is nothing to prevent a small difference existing between one chromosome and the other two of the same kind in the triploid. In this way the number of chiasmata in a certain bivalent in one diploid progeny may be reduced without the total number of chiasmata per cell being necessarily decreased perceptibly.

b. *The trisome carinatum 1.* — At meiosis this forms either 7 bivalents and 1 trivalent or 8 bivalents and 1 univalent. In 10 cells the former alternative was effected in 4 cases, the latter alternative in 6 cases. The chromosomes in a whole cell from this plant are reproduced in Fig. 16 *c*, solitary trivalents in Figs. 16 *d*—*g*. As in the mother plant the commonest type of trivalent here is the V trivalent.

The number of chiasmata in 10 cells was the following:

Number of xx:	0	1	2	3	4	M xx/cell	M term. xx/cell	Term. coeff.
Number of cases:.....	3	20	43	12	2	15,0	3,2	0,213

The number of chiasmata per chromosome was 0,88.

c. *The hypotetraploid carinatum 2.* ($2n = 30$). — The meiosis of this form is characterized, as might have been expected, by the presence of multivalents (Fig. 17 *a*, *b*). The number of multivalents per cell varied between 0 and 5. In 10 cells the following configurations were observed:

1. 2 IV, 2 III, 6 II, 4 I
2. 2 IV, 1 III, 8 II, 3 I
3. 1 IV, 2 III, 10 II
4. 1 IV, 13 II
5. 1 IV, 12 II, 2 I
6. 1 IV, 10 II, 6 I
7. 1 III, 11 II, 5 I
8. 15 II
9. 14 II, 2 I
10. 14 II, 2 I

Of these, No. 3 is especially interesting: in spite of the presence of 2 trivalents this cell did not contain any univalents. This indicates that the

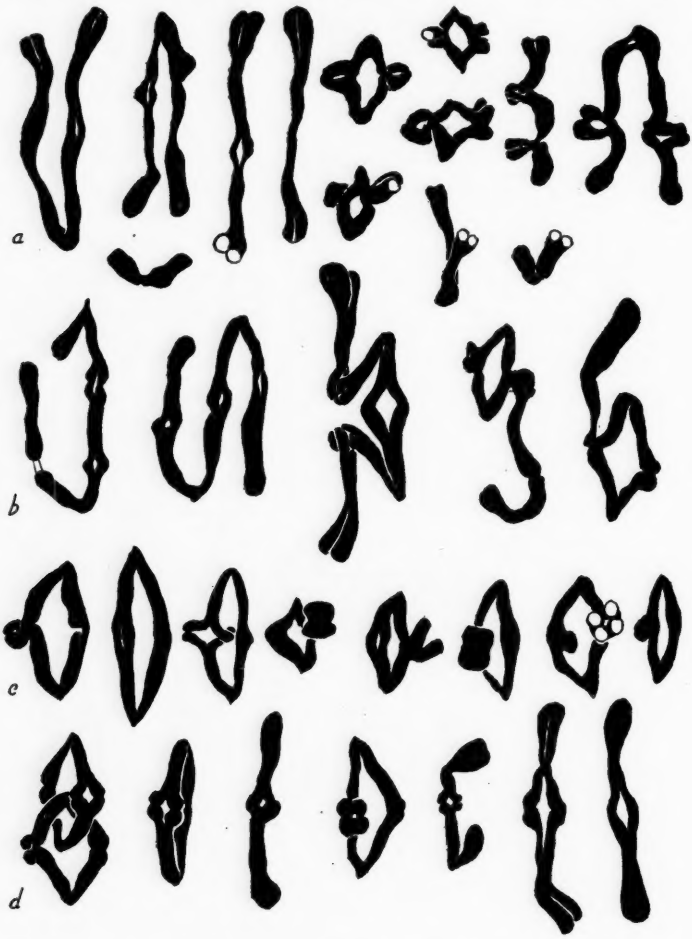

Fig. 17. Meiosis, *a*: *carinatum* 2 ($2n = 30$), *b*: instances of quadrivalents from this plant, *c*—*d*: *A. pulchellum* × *carinatum*, diploid hybrids. — × 2900.

2 chromosomes missing in the tetraploid number must be of different kinds. The constitution of this form of *carinatum* can thus be written:

a1 b1 c1 d1 e1 f1 g1 h1
a2 b2 c2 d2 e2 f2 g2 h2
a3 b3 c3 d3 e3 f3 g3 h3
a4 b4 c4 d4 e4 f4

In the 10 cells analysed the number of chiasmata per cell was:

Number of xx:	21	22	23	24	25	26	27	28	M xx/cell	M term. xx/cell[1]	Term. coeff.
Number of cases:...	2	0	1	3	0	2	1	1	24,4	6,4	0,262

The number of chiasmata in the bivalents present in these 10 cells was:

Number of xx:	1	2	3	4	Total II	Total xx	xx/II
Number of cases:.....	45	53	14	3	115	205	1,8

Thus the number of chiasmata per chromosome was somewhat lower in this form than in diploid forms of *carinatum*.

E. ALLIUM PULCHELLUM × CARINATUM.

a. *Diploid hybrids.* — Meiosis was investigated in 8 such hybrid plants from Nos. 1325 and 1326. All of them had complete bivalent pairing and showed no evidence of hybrid disturbances. The number of chiasmata, however, is on the average lower than in the parents (v. Table 1). The number varies quite considerably from plant to plant, from an average of 11,1 to 15,8 chiasmata per cell, in absolute values from 10 to 18 chiasmata per cell. Whether these variations are due to pure modifications or to genetic differences between the plants cannot be determined, the latter is certainly the more probable, as fixations of different plants made at the same occasion may show considerable differences in the number of chiasmata.

In Figs. 17 *c* and *d* are reproduced plates from the two extreme types, in *e* (1326—96) all the bivalents are ring-shaped, in *f* (1326—40) only 4 bivalents are ring-shaped, 4 being rod-shaped. One case of proximal interlocking is observed in this last-mentioned cell. In conjunction with the varying frequency of chiasmata a varying frequency of univalents is observed. In No. 1325—14 univalents were very common. Their frequency was recorded in a number of cells:

Number of univalents:	0	2	4	Total
Number of cases:	42	30	1	73

It is quite obvious that the *pulchellum* and the *carinatum* genomes are on the whole identical. No cytologically discernible structural differences are found, and in the hybrid between these two species the number of chiasmata has been reduced much less than, for instance, in the examined variety hybrid of *paniculatum*, No. 1094.

b. *The tetraploid hybrid 1326—20.* — This tetraploid is distinguished by the fact that it mainly forms bivalents at meiosis. Compared with other tetraploids of the *paniculatum* group, it has a much lower frequency of multivalents. In 10 analysed cells the following chromosomal configurations were noted:

Number of cases	Configuration
1	3 IV, 9 II, 2 I
1	2 IV, 11 II, 2 I
1	1 IV, 1 III, 12 II, 1 I
1	1 III, 14 II, 1 I
1	13 II, 6 I
3	14 II, 4 I
2	16 II

Fig. 18 *a* shows an analysed plate containing 13 bivalents and 6 univalents, while Fig. 18 *b* shows some quadrivalents chosen at random from several cells. In the 10 cells analysed the frequency of chiasmata was as follows:

Number of xx:	22	23	24	25	26	27	28	31	M. xx/cell	M term. xx/cell	Term. coeff.
Number of cases:...	2	0	2	3	1	0	1	1	25,2	6,9	0,274

The number of chiasmata in the bivalents actually present was the following:

Number of xx:	1	2	3	4	Total II	Total xx	M xx/II
Number of cases:.....	62	52	16	3	133	226	1,7

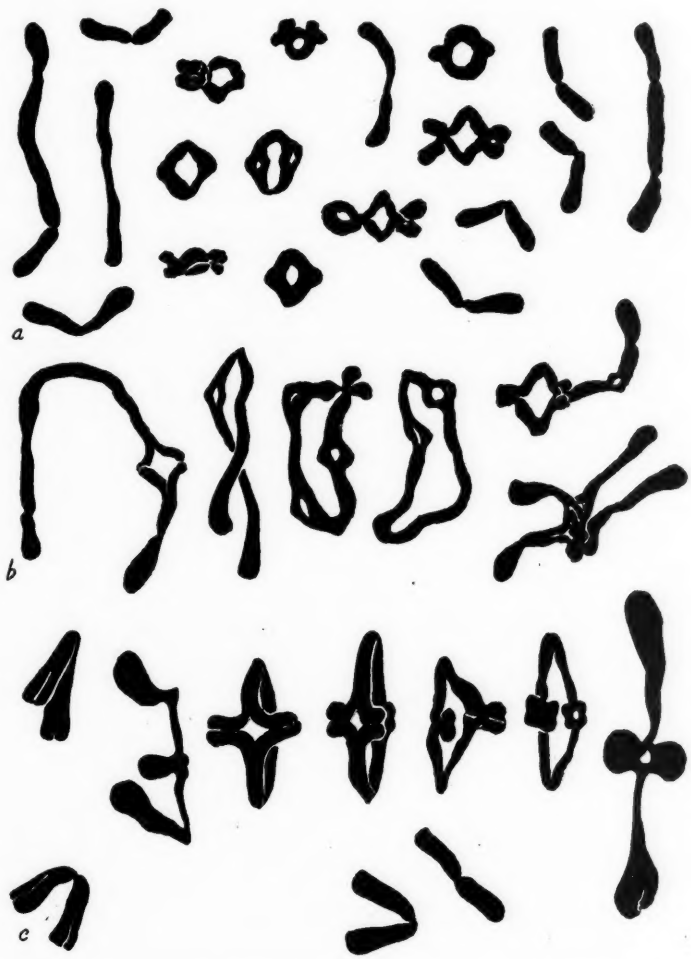

Fig. 18. Meiosis, *a*: *A. pulchellum* × *carinatum*, the tetraploid hybrid, *b*: quadrivalents from this plant, *c*: *A. flavum pumilum roseum* × *carinatum* 4. — × 2900.

As appears, the frequency of chiasmata per chromosome in this tetraploid lies within the limits of the variation of the diploids in this respect.

At anaphase I the univalents always behave in the same manner, they form a secondary equatorial plate and then divide, after which

the half chromosomes pass to the poles. The following numbers of univalents, which were being divided in the equatorial region, were recorded in one slide:

Number of univalents:	0	1	2	3	4	5	6	Total	M
Number of cases:.....	12	13	7	8	3	3	1	47	1,8

At first telophase the univalent halves are almost regularly enclosed in the dyad nuclei. At second division they are again found lagging behind at anaphase. They are now distributed at random between the poles.

F. ALLIUM FLAVUM PUMILUM ROSEUM × CARINATUM.

The type of meiosis which was found to be characteristic of *flavum pumilum roseum* is also predominant in its hybrid with *carinatum* 4. There is homology between the two genomes brought together, only bivalents and univalents occur. In the reproduced plate (Fig. 18 *c*) there appear 4 ring bivalents, 2 rod bivalents and 4 univalents. The number of chiasmata is lower in the hybrid than in either of the parents (v. Table 1), and the commonest type of bivalent is the rod-shaped bivalent with 1 non-terminal chiasma.

III. DISCUSSION AND CONCLUSIONS.

1. POLYPLOIDY AND SPECIES FORMATION.

In the foregoing pages it was shown how the formation of species in nature within the *paniculatum* group consisted in a large measure in the rise of polyploid forms from existing diploids. In one case I succeeded in experimentally synthesising a tetraploid species extensively occurring in nature by crossing two forms of a closely related diploid species. Probably by somatic chromosome doubling the tetraploid was formed already in F_1. In another case I extracted diploid forms from a triploid form found in nature. These diploid forms must be referred to another species likewise occurring in nature, phylogenetically probably the mother species of the triploid in question.

In the *paniculatum* group all polyploid forms examined frequently form a high percentage of multivalents. Consequently they are to a certain extent autopolyploids. In this group, as in several other species of *Allium*, autopolyploidy has thus been shown to be of great importance as an evolutionary factor under natural conditions.

The synthetic *oleraceum* is a new instance of a wild species arisen under experimental control. MÜNTZING (1930, 1932) was the first to succeed in such a synthesis when he obtained a triploid *Tetrahit*-like form »pseudo-*Tetrahit*», in the F_2 of the cross *Galeopsis pubescens* × *speciosa*, which when back-crossed with *pubescens* gave a tetraploid plant »artificial *Tetrahit*». This last-mentioned plant agreed in every respect with the wild species *Galeopsis Tetrahit*.

In the genus *Salix*, HERIBERT NILSSON (1928, 1931), by means of crosses of the wild species *viminalis* and *caprea*, twice obtained progenies which morphologically agree entirely with previously known spontaneous species, viz. the garden species *Salix laurina* and a probably triploid form of *Salix cinerea*.

By crossing *Phleum pratense* with *alpinum*, GREGOR and SANSOME (1930) produced a hexaploid form, which agrees entirely with a form having an extensive geographical distribution in nature within a different area from that of the parent species.

As already mentioned above, I have a parallel case, not yet published, in *Allium Schoenoprasum* to the origin of the new *oleraceum*. In this case the newly formed tetraploid agrees entirely with a tetraploid gigas form of this species known from nature. But there is in this case not such a qualitative difference as the absence or presence of bulbils constitutes in the *paniculatum* group, consequently the tetraploid type of *Schoenoprasum* has not been considered as a species.

It is thus quite obvious that polyploidy is of importance as a species producing factor not only in genetic experiments but also in nature. And it is evident that species which ever since the days of LINNÉ have been looked upon as »good» species have arisen under natural conditions by quantitative variations in entire genomes.

2. POLYPLOIDY AND BULBIL PROPAGATION.

The presence of bulbils in the inflorescences is a very characteristic morphological property in the *paniculatum* group. A certain number of the species occurring in nature are distinguished floristically from one another only by the presence or absence of the bulbils. It is the rule in nature, at least it is so in the material of the *paniculatum* species I have hitherto had occasion to study, that the polyploids are provided with bulbils while the diploids are devoid of bulbils. Things are not so simple, however, in experimental work. The forms dealt with above exhibited, summarily, the following behaviour (+ denotes the presence of bulbils, — denotes the absence):

Species	2n	Presence or absence of bulbils
paniculatum, pulchellum, flavum, flavum pumilum roseum	16	—
carinatum.....	16	4 plants +, 1 plant —
»	17	— (1 plant)
»	19	+ (1 plant, entirely devoid of flowers)
» (wild species).....	24	+
»	30	+ (1 plant)
oleraceum (natural and artificial)	32	+
pulchellum × *carinatum*	16	66 plants +, 86 plants —
» »	17	1 plant +, 1 plant —
» »	32	+ (1 plant)
flavum pum. ros. × *carinatum*.....	16	+ (1 plant)

From the above table it appears that of the pure species (spontaneous and inbred) the diploids have in general no bulbils while plants with higher chromosome numbers than the diploid have bulbils. Exceptions are 4 inbred *carinatum* diploids with bulbils and 1 *carinatum* trisome without bulbils. Of the hybrids the tetraploid plant has bulbils, one of the trisomes has bulbils while the other is without; 66 of the diploids have bulbils while 86 lack them.

It is not easy to find a simple factorial explanation to cover all these facts. The exceedingly variable development of the bulbils indicates that it is a question of quantitative factors, which are moreover very modifiable. The natural *carinatum* 149 employed must have been heterozygous for the bulbil character, and so must have the inbred *carinatum* 4.

The two *paniculatum* forms which gave rise to synthetic *oleraceum* produced 15 diploid plants without bulbils in the same cross. This can be explained factorially by assuming that both parents were heterozygous for a number of complementary bulbil factors, which met in the tetraploid offspring and supplied the bulbil characters. As mentioned in the historical sketch, certain floristic authors have expressed the opinion that true *paniculatum* occasionally bears bulbils.

With regard to the synthetic form of *oleraceum* it seems to me to be more natural to assume that the polyploid stage itself produced the bulbils. Examples are known with respect to which polyploidy has by itself produced entirely new properties in pure autopolyploids, properties not found in the corresponding diploid form. Purely quantitative

chromosomal changes may bring about qualitative changes in the morphology, ecology and standard of reaction of a species. MÜNTZING (1936) gives numerous instances of this phenomenon.

Mention will be made here of only a few cases in which the polyploids, in the same manner as the polyploid forms of *Allium*, exhibit tendencies to change to a vegetative or apomictic mechanism of propagation. In *Nasturtium officinale* (MANTON, 1935) the triploid, on account of its sterility, has been compelled to change to vegetative propagation and has in conjunction therewith attained a different geographical distribution from that of the diploid. A similar tendency is shown by *Biscutella laevigata* (MANTON, 1934) in its tetraploid stage to develop adventitious buds on the roots. In *Festuca ovina* (TURESSON, 1930, 1931), triploids, tetraploids and one hexaploid are viviparous and bulbil-bearing and the degree of vivipary grows with increasing chromosome numbers, diploid forms on the other hand are sexual. That is also the case with regard to *Allium roseum bulbilliferum* (MESSERI, 1930), where the hexaploid is bulbil-bearing while the diploid is sexual. Apomictic races frequently show a higher chromosome number than closely related amphimictic races, e. g. *Chara* (ERNST, 1918), *Sorbus* (LILJEFORS, 1934).

3. THE CONCEPTION OF AUTOGENOMATIC SPECIES.

A characteristic feature in the study of the chromosomes of the *paniculatum* group, both somatic and meiotic, is the great morphological constancy shown by the chromosomes of all the different species and forms, a constancy which is undoubtedly due to a structural identity of the chromosomes in the different forms. In not a single one of the variety or species hybrid plants examined were there any indications of ring- or chain-formation at meiosis or any other evidence of structural differences. The only effect of the differences, which undoubtedly exist, was to reduce the number of chiasmata. This is quite different from the state of things in the other known species hybrid in *Allium*, *A. Cepa* × *fistulosum*.

According to MÜNTZING's definition of autopolyploidy (1936, p. 311: »the genomes of autopolyploids are structurally identical but may be genetically different»), the polyploids in the *paniculatum* group are to be regarded as autopolyploids. In each of the two sub-divisions of the group, *paniculatum—oleraceum* and *pulchellum—flavum—carinatum*, the different species are built up of one structurally identical genome present in different numbers of repetitions and provided with a varying

assortment of genes. Whether the genomes *between* these two subdivisions are also structurally identical I have been unable to investigate, as they have hitherto been separated by a barrier of sterility.

In conformity with the term autopolyploidy it would, in my opinion, be appropriate to introduce a designation for different diploid species which have been proved by crossing experiments to consist of structurally identical genomes but which may be genetically different, viz. the designation *autogenomatic* species. In crossing, such species frequently show a reduced number of chiasmata but never such pairing abnormalities as amphibivalent formation, geminus asymmetry, etc. On the appearance of amphidiploidy in autogenomatic species autopolyploidy arises (*sensu* MÜNTZING). The difference between the conception of autogenomatic and the old terms homogenomatic and isogenomatic (WINKLER, 1920) is obvious.

The term allogenomatic, which denotes diploid species built up of structurally dissimilar genomes, is of less importance in practice.

4. CHIASMA FORMATION.

The number of chiasmata at meiosis is important for the interpretation of chromosome homologies only when good chromosome pairing is present. Good pairing must be interpreted as good chromosome homology, whereas poor pairing need not in itself imply poor homology, for the presence of a single asynaptic gene may suppress the entire meiotic pairing in an organism.

In the diploid *paniculatum* hybrids examined the number of chiasmata was reduced as compared with inbred or free-flowering forms of the species. The following chiasma values are available (cf. Table 1):

Form	xx/chromosome	Reduction
paniculatum 259	1,17	27,3 %
» 1094 (a variety hybrid)	0,85	
carinatum 4	1,00	31,7 %
flavum pum. roseum	0,64	
their hybrid	0,56	
pulchellum	0,99	15,0 %
carinatum 4	1,00	
average of their hybrids	0,85	

In an ideal material, where all special pairing genes would be identical in different forms and where the gene differences present

would be scattered at random over the chromosome set, the reduction % figures may express the degree of difference in the gene complement between the genomes brought together in the hybrid. These species of *paniculatum* by no means constitute such an ideal material. The very variable chiasma values in the cross *pulchellum—carinatum* indicate, for instance, a re-combination of genes affecting the pairing.

The special type of meiosis showed by *A. flavum pumilum roseum*, a type which was dominant in the crossing of that form with *carinatum* 4, is a further example of the genetic control of meiosis of a similar nature to the localisation of chiasmata occurring in certain *Allium* species, although less striking in its effect. The localisation of chiasmata showed, however, a recessive inheritance (EMSWELLER and JONES, 1934).

With regard to the number of chiasmata in the polyploids as compared with the corresponding number in the diploids the following data are available:

Form	xx/chromosome
Diploid variety hybrid of *paniculatum*	0,85
Tetraploid sister plant, synthetic *oleraceum*	0,97
Diploid *carinatum*	0,99, 1,00
Trisomic »	0,88
Triploid »	0,85
30-chromosome *carinatum*	0,90
Diploid *pulchellum* × *carinatum*	0,72—0,99
Tetraploid » × »	0,85

As the number of plants examined is so small and as the figures refer to only one fixation of each plant, no definite conclusions can of course be drawn. On the whole the number of chiasmata per chromosome agree very well between diploids and other forms. The whole of the existing variation lies between 0,72 and 1,00 chiasmata per chromosome. In one case, i. e. the synthetic *oleraceum,* the tetraploid shows a much higher number of chiasmata as compared with the diploid sister plants. This may quite simply be due to the occurrence of autosyndesis in the tetraploid. As a result of the doubling of the somatic chromosomes 16 pairs of chromosomes are present, within which the two homologies are completely identical and can thus show maximal pairing.

In *Allium carinatum* all the 3 forms with chromosome numbers above 2x show a lower number of chiasmata per chromosome than the diploids. In this case we should call attention to a possibility suggested by the agreement of these chiasma values with the viability curves

drawn for other *Allium* species just for the range 2x—4x: Perhaps the general viability of the cells plays a part in the pairing of the chromosomes, as it has been shown to do in such properties as pollen fertility, self-fertility, etc. (LEVAN, 1936 a, b).

The terminalisation of the chiasmata was rather low in the entire *paniculatum* group. The terminalisation coefficient (= the number of terminal chiasmata : the total number of chiasmata) in the various forms was as follows:

paniculatum	0,18, 0,34
synthetic *oleraceum*	0,25
pulchellum	0,21, 0,22
flavum	0,26
flavum pumilum roseum	0,12
carinatum, diploid	0,14, 0,15
» , trisomic	0,21
» , triploid	0,26
» , hypotetraploid	0,26
pulchellum × *carinatum*, diploid	0,10—0,24 (M: 0,19)
» × » , tetraploid	0,27
flavum pumilum roseum × *carinatum*	0,09

As appears, the variation in all forms is 0,09—0,34. A characteristic feature was that the terminalisation in V-shaped chain trivalents was strikingly high, most certainly higher on the average than in the bivalents in the same cell. This has contributed to the higher terminalisation coefficients in triploids and tetraploids as compared with the diploids. The relatively low terminalisation in the synthetic *oleraceum* was computed on bivalents only (v. p. 352) and is therefore not comparable with other values. The terminalisation coefficient of the diploid forms is on an average 0,19, while forms with more chromosomes than 2x have the average value of 0,25.

As already mentioned, the shape of the bivalents play a rôle in the effect of the terminalisation. This effect is greatest in ring-shaped bivalents, less in rod bivalents. Therefore we find a positive correlation between number of chiasmata and terminalisation coefficient. Those forms in the *paniculatum* group which have the lowest numbers of chiasmata per cell, viz. *flavum pumilum roseum* and its hybrid with *carinatum*, also show the lowest terminalisation coefficient.

IV. SUMMARY.

1. The present work is mainly a cytological study of the following species of *Allium*: *paniculatum*, *pulchellum*, *flavum* (2n = 16), *carinatum* (2n = 24) and *oleraceum* (2n = 32). Together these species constitute the *paniculatum* group belonging to the Section *Macrospatha*.

2. A historical-taxonomical orientation is given of these species together with their more important subspecies and forms (I, 1).

3. Then follows a list with a short description of the forms in the *paniculatum* group cultivated by the author. These forms make up the basis of the present investigation (I, 2).

4. A cross between two extreme types of *A. paniculatum* gave, by doubling of the chromosome number, a tetraploid form belonging to *A. oleraceum* (I, 3, A).

5. By self-fertilisation *A. carinatum* gave rise to 5 plants with somatically 16, 17, 19 and 30 chromosomes. The 17-chromosome plant, which lacked bulbils and morphologically agreed with *A. pulchellum* in other respects, was especially interesting (I, 3, B).

6. Among the self-fertilized progeny of a bulbil-bearing 16-chromosome I_1 plant after spontaneous *carinatum* there was one 16-chromosome plant without bulbils. This plant had thus completed the transgression from *A. carinatum* to *A. pulchellum* (I, 3, B).

7. A 16-chromosome *A. carinatum* was easily crossed with *A. pulchellum* and gave abundant progeny. This progeny contained, in addition to plants with 16 chromosomes, 2 plants with 17 chromosomes and 1 plant with 32 chromosomes. The segregation for the character presence—absence of bulbils was observed, 66 plants having bulbils while 86 lacked them (I, 3, C, a).

8. A 16-chromosome *carinatum* was also crossed, although with some difficulty, with a form of *flavum*, *flavum pumilum roseum*. Only one F_1 plant was obtained. This plant had characters from both parents (I, 3, C, b).

9. A simple and rapid method of producing slides of pollen mother cells of the *paniculatum* species is described (II, 1).

10. A description is given of the somatic chromosome morphology of the species (II, 2).

11. Meiosis in the spontaneous species as well as in the experimental derivatives is described. 10 complete cells of each type were analysed with respect to chiasmata conditions. The results of this analysis are given in Table 1. Judging from the pairing of chromosomes *A. oleraceum*

consists of 4 *paniculatum* genomes and *A. carinatum* of 3 *pulchellum* genomes (II, 3).

12. Polyploidy as a factor in species formation in the *paniculatum* group is discussed (III, 1).

13. The relation of the bulbil character to the chromosome number is investigated (III, 2).

14. The term *autogenomatic* is suggested to designate different genomes which are experimentally shown to be identical in their chromosomal structure, although not necessarily identical in their gene assortment (III, 3).

15. The chiasma conditions at meiosis of the different forms are discussed from different aspects: *1.* the number of chiasmata as a measure of chromosome homologies, *2.* as a function of special pairing genes, *3.* the relation between the number of chiasmata in diploids and in corresponding polyploids, *4.* the terminalisation of chiasmata within the *paniculatum* group (III, 4).

Hilleshög, Landskrona, Sweden, October 1936.

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MULTIPLE ALLELS AND POLYMERIC FACTORS IN GALEOPSIS

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As demonstrated in detail in a previous publication (MÜNTZING, 1930 a) several of the species belonging to the genus *Galeopsis* are very polymorphic. This polymorphism is chiefly of a quantitative nature, but there are also numerous differences which are more qualitative, and are possible to analyse genetically. The results of a factorial analysis in *G. Tetrahit* and *bifida* have already been summarized (l. c. pp. 192—215). Since that account was written, some new data have accumulated, which need a short description. These data are limited to *G. Tetrahit* and *pubescens*. In the latter species intraspecific inheritance has not previously been studied.

I. INHERITANCE OF HAIRINESS AND FLOWER COLOUR IN G. PUBESCENS.

Like the majority of other *Galeopsis* species, *G. pubescens* may easily be self-fertilized, and the present material of this species (chiefly obtained from Austria) consists of eight different pure lines. These lines have been in culture since 1927 or 1928 and seem to be completely homozygous. The lines differ strikingly in vegetative characters as well as in flower colour, but within each line there is complete uniformity. The results recorded below are confined to crosses between the lines P—A, P—B, P—C and P—D. Of these lines P—A and P—C are red-flowered (of a somewhat different shade), the other two lines (P—B and P—D) have yellow flowers. The flower of P—B may be distinguished from that of P—D by the absence of a spot of orange yellow, which is present on the side laps of the P—D flower.

The hairiness of these lines is strikingly different. As indicated by the species name, *G. pubescens* is characterized by the presence of soft (unicellular) hairs. In addition to these hairs there may also be multi-cellular bristle hairs in varying quantities. That is the case in three of the lines examined (P—A, P—B and P—D), which represent three different degrees of bristle hairiness. As is evident from the stems

reproduced in Fig. 1, P—A (No. 2) has almost exclusively soft hairs, but especially on the upper internodes there is also a small proportion of bristle hairs. In the P—B line (Fig. 1, No. 3) there are plenty of bristle hairs on the upper as well as on the lower internodes, and in P—D, finally, the bristle hairiness is extreme (Fig. 1, No. 4). In contrast to these three lines, having bristle hairs in varying quantities, the line P—C (Fig. 1, No. 1) has only soft hairs, the internodes being quite smooth and devoid of the stiff bristle hairs.

These four lines were crossed in the six possible directions, the following results being obtained.

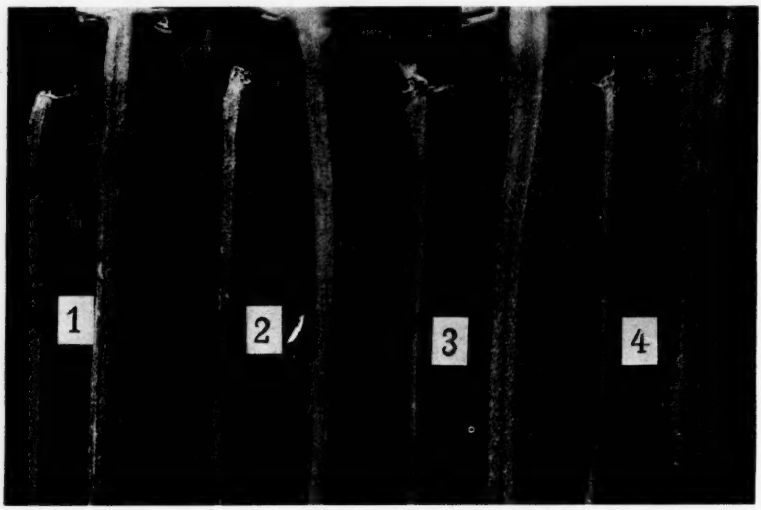

Fig. 1. Upper and lower internodes from four pure lines of *Galeopsis pubescens*. No. 1 (P—C) has only smooth hairs, Nos. 2—4 (P—A, P—B and P—D respectively) represent different degrees of bristle hairiness.

1. CROSS 1. P—A × P—B.

Phenotypical differences. — P—A has red flowers and a quite low degree of bristle hairiness, P—B has yellow flowers and a rather pronounced bristle hairiness.

Segregation. — In F_1 the flower colour was intermediate or with some prevalence of the yellow colour. The higher degree of bristle hairiness was dominant. In F_2 there was dihybrid segregation, but since the heterozygous flower colour could be distinguished there were six different phenotypic categories. Of the 95 F_2 plants, 37 had flowers

of the F_1 type and abundant bristles, 15 had yellow flowers and abundant bristles, 13 had F_1 flowers and few bristles, 8 yellow flowers and few bristles, 20 red flowers and abundant bristles, 2 red flowers and few bristles. The expected ratio is 6 : 3 : 2 : 1 : 3 : 1. According to this ratio the expected values per 95 are $35{,}63 : 17{,}81 : 11{,}88 : 5{,}94 : 17{,}81 : 5{,}94$. A comparison between the observed and expected values, according to the χ^2 method (FISHER, 1930), gives a χ^2 value of $4{,}20$, which corresponds to a P value situated between $0{,}70$ and $0{,}50$.

The agreement between expectation and observation is evidently quite good. Thus, the degree of bristle hairiness and the flower colour are controlled by two different and independent factors. Taking each character separately, it was found that the ratio of plants with abundant and few bristle hairs was equal to 72 : 23. Concerning flower colour, 23 plants had yellow flowers, 50 were intermediate (F_1 type) and 22 were red-flowered. The former ratio evidently corresponds to 3 : 1, the latter to 1 : 2 : 1.

2. CROSS 2. P—A × P—D.

Phenotypical differences. — P—A has red flowers and a low degree of bristle hairiness, P—D has yellow flowers and an extreme degree of bristle hairiness.

Segregation. — This cross is parallel to Cross 1, but in the present case the difference in hairiness is greater, and therefore classification was still more easy than in Cross 1. As before, the F_1 plants were intermediate in flower colour and the high degree of hairiness was dominant. In F_2 286 individuals were classified and found to belong to the following categories: 102 with F_1 flowers and abundant hairs, 63 with yellow flowers and abundant hairs, 44 with F_1 flowers and few bristle hairs (like the P—A line), 17 with yellow flowers and few hairs, 45 with red flowers and abundant hairs and 15 with red flowers and few hairs. As in Cross 1 the expected ratio is 6 : 3 : 2 : 1 : 3 : 1 per 16 and $107{,}25 : 53{,}63 : 35{,}75 : 17{,}88 : 53{,}63 : 17{,}88$ per 286. A comparison between the observed and expected numbers gives the χ^2 value of $5{,}70$. This corresponds to a P value situated between $0{,}50$ and $0{,}30$.

Thus, also in this cross the agreement between expectation and observation is quite good. Taking each of the two differences separately, 222 F_2 plants had abundant bristle hairs and 80 were of the P—A type. In regard to flower colour, 80 plants were yellow, 146 were of the F_1 type and 60 were red-flowered. Without further calculations it is obvious that these ratios correspond to 3: 1 and 1 : 2 : 1 respectively.

3. CROSS 3. P—A × P—C.

Phenotypical difference. — P—C has quite smooth stems (no bristle hairs), P—A has a low degree of bristle hairiness.

Segregation. — With regard to the bristles, the F_1 plants were similar to P—A. In F_2 classification was impossible (on account of the slight difference between the parent lines), but no plants had a stronger degree of hairiness than P—A. About one fourth of the plants had quite smooth stems, but these individuals could not be distinguished with certainty from those having a quite low degree of hairiness.

4. CROSS 4. P—B × P—D.

Phenotypical differences. — Both lines have bristle hairs, but the degree of hairiness is higher in P—D than in P—B (cf. Fig. 1, Nos. 3 and 4). Further, P—D, in contrast to P—B, has a spot of orange yellow on the side laps of the flowers.

Segregation. — Since P—B as well as P—D were found to give monohybrid segregation with P—A as regards the degree of bristle hairiness, it was of interest to see if these two lines had allelic or different genes for hairiness. The first alternative was found to be true. The F_1 plants, as expected, had abundant bristle hairs and the same thing was true of all the 278 F_2 plants raised. The ratio 278 : 0 cannot represent a 15 : 1 segregation ($P = < 0{,}01$ and $D/m = 4{,}55$). — Most of these F_2 plants were examined at an early stage, since the differences in hairiness are clearly visible already at the seedling stage. Only 26 plants were transplanted into the field. All of these had yellow flowers, as expected, but 20 had the orange spot on the side laps and 6 had no spot. Thus, this difference, which was not studied further, is probably caused by a single gene. The important point in this cross, however, is the fact that the two lines were found to have allelic hairiness factors.

5. CROSS 5. P—C × P—B.

Phenotypical differences. — P—B has yellow flowers and abundant bristle hairs, P—C has red flowers and no bristle hairs.

Segregation. — This cross may be compared with P—A × P—B, but in the present case the difference in hairiness between the parent lines is still more clear-cut, P—C having quite smooth stems, devoid of all traces of bristle hairs. In F_1 there was a dominance or at least prevalence of bristle hairiness and the flowers were of the usual intermediate type, which, however, is more like the yellow than the red

flower colour of the parents. In F_2, 90 plants were examined, and of these 78 were hairy and 12 smooth. Assuming monohybrid segregation, the expected ratio is 67,5 : 22,5. The χ^2 value in this case will be 6,53, giving a P value situated between 0,02 and 0,01. Thus, there is most probably a true deficit of recessive plants. — As regards flower colour 19 F_2 plants were yellow, 16 red and 55 of the F_1 type. The expected ratio is 1 : 1 : 2 or 22,5 : 22,5 : 45,0. The χ^2 value in this case will be 4,64, corresponding to a P value intermediate between 0,10 and 0,05. Thus, it is rather probable that the segregation of flower colour is also disturbed by some unknown influences. — Combining the two segregating differences, the usual six categories were found to be represented by the following numbers: 48 hairy F_1 types : 15 hairy and yellow : 7 smooth and F_1 : 4 smooth and yellow : 15 hairy and red : 1 smooth and red. According to the theoretical ratio 6 : 3 : 2 : 1 : 3 : 1 the values expected per 90 are 33,75 : 16,88 : 11,25 : 5,63 : 16,88 : 5,63. The agreement between observation and expectation is not good, especially with regard to the first class. A calculation of χ^2 gave the value 12,37, which corresponds to a P value intermediate between 0,05 and 0,02.

Thus, though it is possible that the observed values correspond to a normal dihybrid segregation it is rather probable that some disturbing complication is at work. In order to study this more closely and especially as there seemed to be an excess of hairy over smooth plants in this cross, the following six F_3 families were raised.

A. TWO PROGENIES FROM PLANTS WITH F_1 FLOWERS AND BRISTLE HAIRS.

One of these progenies, consisting of 29 plants, was constant in hairiness and segregated in 5 yellow : 15 F_1 : 9 red as regards the flower colour. The other mother plant gave segregation of the F_2 type, the following numbers being observed: 14 hairy and F_1 : 4 hairy and yellow : 5 smooth and F_1 : 2 smooth and yellow : 6 hairy and red : 5 smooth and red. Two hairy plants died before flowering. Thus, the flower colour segregated according to the ratio 6 yellow : 19 F_1 : 11 red, and with regard to the other difference, 26 plants were hairy and 12 smooth.

B. THREE PROGENIES FROM PLANTS WITH RED FLOWERS AND BRISTLE HAIRS.

All members of these progenies were red-flowered, as expected, and one progeny seemed to be constant also in hairiness (20 individuals). The other two progenies segregated in 33 hairy : 5 smooth and 26 hairy : 3 smooth respectively.

C. ONE PROGENY FROM A PLANT WITH F_1 FLOWERS AND SMOOTH STEMS.

This progeny consisted of only 4 individuals, which were all smooth like the mother plant. Two of these plants had F_1 flowers, two were red-flowered.

According to the data just given three of the F_3 families segregate in hairy—smooth, the ratios observed being 26 : 12, 33 : 5 and 26 : 3. These ratios correspond to the χ^2 values 0,88; 2,84 and 3,32 respectively. In the first case the P value will be intermediate between 0,50 and 0,30, in the latter two progenies P will be situated between 0,10 and 0,05. Thus, the F_3 families under discussion may all represent uncomplicated monohybrid segregations, though the agreement between observation and expectation is rather poor in two of the three progenies. However, the sum of the χ^2 values of F_2 and the three F_3 families is equal to 13,57. As there are four degrees of freedom the corresponding P value is smaller than 0,01. Hence it is practically certain that in this cross the monohybrid segregations hairy : smooth are often disturbed by some complication, causing a deficit in the recessive category. As in many other cases, this is probably a question of differential viability. This hypothesis, however, needs further investigation. Nevertheless it is highly probable that also in this cross the difference in hairiness is caused by a single gene.

6. CROSS 6. P—C × P—D.

Phenotypical differences. — P—C has smooth stems and red flowers, P—D has an extreme bristle hairiness and yellow flowers.

Segregation. — The cross is quite analogous to Cross 5, but the hairiness of P—D is more extreme than that of P—B. As usual the F_1 plants had bristle hairs and intermediate flower colour. In F_2, 126 plants were raised and were found to segregate in 91 individuals with bristle hairs and 35 with smooth stems. This ratio evidently corresponds to a typical monohybrid segregation. With regard to the flower colour the ratio 96 yellow and F_1 types: 30 red was obtained. In this case the pure yellow and more intermediate colour of F_1 type were not distinguished, but it is obvious, as expected, that the total number of such plants is about three times as great as the number of red-flowered F_2 plants. As usual the two factors were independent of each other. the following dihybrid ratio being obtained: 67 hairy, yellow (or F_1) : 29 smooth, yellow (or F_1) : 24 hairy, red : 6 smooth, red. The expected values per 126 are 70,88 : 23,63 : 23,63 : 7,88. The χ^2 value is 1,89 and P is thus situated between 0,70 and 0,50.

7. CONCLUSION.

A. FLOWER COLOUR.

From the crosses described above it is evident that the difference between the yellow and red flower colour is mainly caused by one gene. Other modifying factors with a slight effect are certainly also involved, since at least the two red-flowered lines differ in the shade of their colour and since the F_2 generations were really somewhat more variable than expected on a single factor basis. — The flower colour in F_1 is rather intermediate but more like the yellow than the red colour. At any rate it was easier to pick out the red-flowered F_2 plants than those with purely yellow colour. Thus the factor for yellow flower colour almost acts as an inhibiting factor on the red colour. Therefore it may be correct to write YY = yellow and yy = red.

B. HAIRINESS.

As demonstrated above, the crosses between smooth and hairy and between low and high degree of bristle hairiness give all monohybrid segregations. Further, the cross between the lines representing the two highest degrees of bristle hairiness gave no smooth segregates in F_2. Thus, it is evident that in this material *the degree of bristle hairiness is controlled by a system of multiple allels.* The gene involved must occur in four different conditions. As one of the lines has only smooth hairs, in contrast to the other three lines which represent different degrees of bristle hairiness (Fig. 1), it may be convenient to use the symbols b, B_1, B_2 and B_3 for the lines P—C, P—A, P—B and P—D respectively.

II. INHERITANCE OF HAIRINESS AND FLOWER COLOUR IN G. TETRAHIT.

1. HAIRINESS.

The pure lines of the tetraploid species, *G. Tetrahit*, have also smooth hairs and bristle hairs, but the smooth hairs are less abundant than in *G. pubescens*. The degree of bristle hairiness is highly variable, some lines being extremely hairy (Fig. 2, No. 5) while other lines are almost smooth (Fig. 2, No. 2). In crosses between lines, representing different degress of bristle hairiness, classification in F_2 and following generations was impossible. However, in none of the crosses studied (24 crosses described in the 1930 a-paper and 17 additional crosses discussed below) was there any segregation of F_2 plants without bristle hairs. Thus, all the 19 lines involved must have at least one gene for bristle hairiness (or allels of this gene) in common.

An excellent possibility to analyse the genetical basis of bristle hairiness in *G. Tetrahit* was obtained by the introduction of a new *Tetrahit* line, completely devoid of bristle hairs as well as smooth hairs. For this »*glabra*» line (Fig. 2, No. 1) I am much indebted to the late Dr. K. TJEBBES, who collected seeds of this type during a journey in Italy. From these seeds four plants were obtained in 1932, and of these plants three were complete *glabra* types without the slightest trace of bristle hairs or smooth hairs. Only glandular hairs were present (as in all other *Galeopsis* types) but these seemed to develop slowly

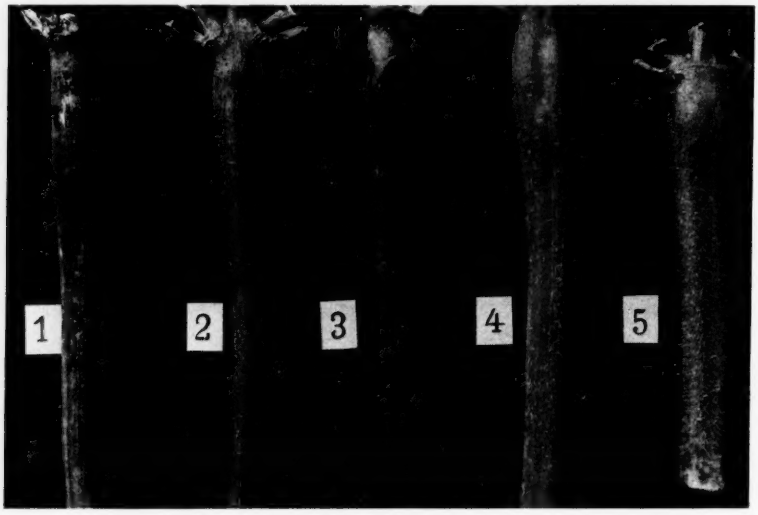

Fig. 2. Internodes from five pure lines of *Galeopsis Tetrahit*. No. 1 (the »*glabra*» line) is quite glabrous, Nos. 2—5 (T—F, T—D, T—I and T—L respectively) show different degrees of bristle hairiness.

and appear late. The fourth plant differed from the other three in having a low degree of bristle hairiness. Otherwise it was similar in all respects to the sister plants. Unfortunately progeny was not raised from this deviating plant but only from one of the other three plants representing the new and interesting type. — This *glabra* type has remained absolutely constant in the subsequent four generations and certainly represents a pure line. This line has been crossed with several other *Tetrahit* lines, and in four of these crosses F_2 generations have

been studied. — All F_1 plants have been hairy and thus the *glabra* character is recessive. In F_2 the following ratios were obtained [1].

Cross	*Observed values*	*Expectation*	χ^2	P
T—E × *glabra*	430 hairy : 43 smooth	443,44 : 29,56	6,47	$0{,}02 > P > 0{,}01$
T—D × »	816 » : 46 »	808,13 : 53,88	1,23	$0{,}30 > P > 0{,}20$
T—L × »	105 » : 13 »	110,63 : 7,38	4,57	$0{,}05 > P > 0{,}02$
T—I × »	53 » : 3 »	52,50 : 3,50	0,08	$0{,}80 > P > 0{,}70$
Total	1404 » : 105 »	1414,69 : 94,31	1,29	$0{,}30 > P > 0{,}20$

From the above values it is evident that the segregations correspond to the ratio 15 : 1. Thus, the *glabra* line is a double recessive and *the hairiness of the ordinary Tetrahit line is conditioned by two dominant polymeric factors.*

In some of the crosses, however, the segregation ratios seem to be somewhat disturbed. In the crosses T—E × *glabra* and T—L × *glabra* there is an excess of recessive plants, the P value in the first cross being situated between 0,02 and 0,01, and in the second cross between 0,05 and 0,02. (A calculation of the D/m values gave the corresponding figures 2,5 and 2,1 respectively). In the second cross (T—D × *glabra*), however, the agreement between expectation and observation is quite good and the number of individuals is rather high. In the fourth cross (T—I × *glabra*) the number of individuals is somewhat too low to allow a calculation of χ^2 and P. Nevertheless it is obvious that the ratio 53 : 3 may correspond to 15 : 1.

If the figures of all four F_2 generations are added, this gives a total of 1404 hairy : 105 *glabra* types. These numbers correspond very well to the theoretical 15 : 1 ratio, the χ^2 value being 1,29 and P intermediate between 0,30 and 0,20. It is more correct, however, to add the χ^2 values of the separate crosses. In this way the value 12,35 is obtained. As there are four degrees of freedom, this gives a P value situated between 0,02 and 0,01. If the fourth cross (T—I × *glabra*) is excluded, on account of the low number of individuals, the χ^2 of the other crosses will be 12,27 and the corresponding P value smaller than 0,01. — Thus, though in the main the crosses are dimeric, there is most probably some complication, causing greater deviations from the 15 : 1 ratio than can be explained by chance distribution alone. This disturbance is certainly not represented by erroneous classification, since the difference between the two categories is quite clear-cut already at the seedling stage. As will be further discussed below, the most plausible cause of the

[1] Most of this material was classified without difficulty at the seedling stage.

disturbed ratios is rather the frequent occurrence of meiotic irregularities and partial sterility in the F_1 hybrids of intraspecific crosses in *G. Tetrahit*.

2. FLOWER COLOUR.

A. SEGREGATION OF FLOWER COLOUR IN THE GLABRA CROSSES.

Like several other *Tetrahit* lines the *glabra* type has dominant white flower colour (cf. MÜNTZING, 1930 a, p. 193). As described above, most of the material in the *glabra* crosses was classified with regard to hairiness already at the seedling stage. In the cross T—E × *glabra* all the F_2 plants were discarded after classification, but in the other crosses some or all of the plants were transplanted to the field, and the flower colour of the full grown plants was observed with the following result.

a. *T—D × glabra.*

With regard to flower colour the T—D line has the constitution *aa* h_1 h_1 *rr II* (MÜNTZING, 1930 a, p. 202), which in the main implies that it is recessive to red as well as dominant white colour. As expected from the appearance of the *glabra* flower, the purely white colour of this line proved to be quite dominant over the T—D colour (upper lip faintly violet, buds slightly flesh-coloured, the basis of the side laps violet-blue), and in F_2 there was monohybrid segregation, the numbers obtained being 150 dominant white: 48 plants with T—D colour. This ratio is almost ideal and needs no statistical test. — From this result it is clear that the *glabra* line has the formula *aa HH rr*. Further, as the network on the middle lap is of the same weak type as in the lines T—A and T—I, the *glabra* line has probably also the factor *i* in contrast to T—D, which has a marked network and the allel *I*. This difference was also observed to segregate in F_2, but classification could not be done with certainty.

b. *T—L × glabra.*

The T—L line is red-flowered, having the constitution *aa* h_1h_1 *RR*. According to the previous cross the *glabra* line carries the factors *aa HH rr*. As in other similar cases the *H* factor of the white-flowered line proved to be completely dominant in F_1. In F_2 there should be segregation according to the ratio 12 dominant white : 3 red : 1 T—D type, but the numbers actually observed were 99 white : 13 red. A comparison between these values and the expected ratio (84 : 21 : 7) gave an χ^2 value of 12,73. The corresponding P value is smaller than

0,01, and thus it is evident that the deviations from the expected ratio are significant.

c. *T—I* × *glabra.*

Also in this cross, which is analogous to the preceding one (with regard to the *H* and *R* factors), the expected dihybrid segregation was found to be much disturbed. F_1 was dominant white and in F_2 there were 33 dominant white and 23 red individuals. It is immediately obvious that these numbers do not agree at all with the expected 12 : 3 : 1 ratio.

The causes of these deviations in the two last-mentioned crosses will be further considered below. At present it should only be observed that regular segregation of flower colour was only found in the cross T—D × *glabra*, in which segregation in hairy : smooth was also quite regular. In the cross T—L × *glabra*, on the contrary, the ratios for flower colour as well as for hairiness were rather divergent (cf. above p. 379). With regard to the third cross (T—I × *glabra*), finally, it may be mentioned already now that it gave a rather sterile F_1 generation, the average percentage of good pollen grains being as low as 68.

B. VERIFICATION OF THE FACTORIAL CONSTITUTION OF THE LINES T—E, T—F, T—G AND T—L.

The crosses described under this heading do not contribute anything essentially new to the factorial analysis of the species, but they more or less verify the conclusions reached from previous crosses (MÜNTZING, 1930 a, p. 202).

a. *T—F* × *T—G.*

Phenotypical differences. — With regard to flower colour the phenotypical differences between T—F and T—G are very slight, but the T—F flower is »diffuse», the T—G colour »distinct», i. e. in T—G the red colour of the side laps is not evenly, »diffusely», distributed all over the lap as in T—F, but is concentrated to a basal spot.

Factorial differences. — T—F has the constitution *aa hh RR*, T—G is *AA* h_1h_1 *rr*.

Segregation. — The F_1 plants were diffusely red, and among the 134 F_2 plants the following categories and frequencies were observed: 92 red diffuse : 34 red distinct : 7 T—D types : 1 T—C. The segregation is trihybrid and the theoretical ratio per 64 is 48 red diffuse : 12 red distinct : 3 T—D : 1 T—C. Calculated per 134 the expected ratio is 100,50 : 25,13 : 6,28 : 2,09. The agreement between observed and expected values is quite good ($\chi^2 = 4{,}50$; $0{,}30 > P > 0{,}20$). Thus, from this

cross as well as from other previous crosses it is evident that the two lines have different factors for red flower colour and, further, since T—D as well as T—C plants appear, the lines must indeed differ also with regard to the $h_1 - h$ allels.

b. *T—C* × *T—E*.

Phenotypical differences. — T—E has light red flower colour of the diffuse type, T—C represents the recessive white flower colour.

Factorial differences. — T—C is *rr*, T—E R_1R_1.

Segregation. — The F_1 plants were light red and in F_2 there was segregation in 27 red : 8 T—C. This ratio is evidently in agreement with the expected monohybrid segregation, and since no T—D plants appeared, it also demonstrates that both lines have the recessive factor *h*.

c. *T—E* × *T—F*.

Both these lines are red-flowered, but the red colour is darker in T—F than in T—E. Judging from other crosses, the red factors in T—E (R_1) and T—F (R) represent different members of the same system of multiple allels. This was, indeed, verified by the observation that 80 F_2 plants raised were all red-flowered (like the parents and F_1). The probability that 80 : 0 may represent a 15 : 1 segregation is less than 0,01. The χ^2 method gives a P value intermediate between 0,05 and 0,02, but according to the tables given by BONNIER and TEDIN (1936) the P value must be still lower, the probability that 71 : 0 represents a 15 : 1 distribution being as low as 0,0102. Since the number of individuals (80) in the present case was somewhat higher than 71, the P value must be lower than 0,0102.

d. *T—B* × *T—L*.

Phenotypical difference. — T—B is dominant white, T—L is red-flowered with diffusely coloured side laps (like all other red-flowered lines with the exception of T—G).

Factorial differences. — T—B is *aa HH RR* and T—L, presumably, *aa* h_1h_1 *RR*.

Segregation. — The F_1 plants were dominant white and in F_2 there was segregation in 77 dominant white : 21 red (diffuse). This ratio may evidently correspond to the expected monohybrid segregation ($0{,}50 > P > 0{,}30$), but the number of individuals is not sufficient to prove that the *R* factors of the two lines are really identical or allelic. Admitting the possibility that the segregation represents a 48 : 15 : 1 ratio it is nevertheless more probable that it is monohybrid, as expected.

Further evidence that the factors for red colour are allelic is represented by the fact that the *H* factor of T—B was completely dominant over the red factor in T—L. From other crosses it is known that *H* is completely dominant over the red factors in the system $r - R_1 - R$ but not completely dominant over the other red factor *A*, present in the line T—G.

C. T—A × T—G.

Phenotypical differences. — T—A has dominant white flower colour and a very weak network on the middle lap, T—G has red flowers of the »distinct» type and a marked network.

Factorial differences. — According to previous crosses (see the 1930 a-paper) T—A has the constitution *aa HH rr ii* and T—G *AA* h_1h_1 *rr II.*

Segregation. — The F_1 hybrids had pink flowers, as is always the case when the factors *H* and *A* are brought together. Further, the clear network of the T—G parent was found to be dominant. In F_2 eight different categories are to be expected, but only six of them were represented, the following frequencies being observed: 90 pink, clear network : 12 pink, weak network : 34 red, clear network : 0 red, weak network : 28 dominant white, clear network : 3 dominant white, weak network : 10 T—D colour, clear network : 0 T—D colour and weak network.

Considering flower colour first, it is obvious that the observed ratio 102 pink : 34 red : 31 dominant white : 10 T—D corresponds very well to the expected dihybrid ratio (9 : 3 : 3 : 1). The χ^2 value is only 0,32 and P intermediate between 0,98 and 0,95. — The segregation of the network is more difficult to understand. In several other crosses, involving the T—A line, the difference between weak and clear network was found to give monohybrid segregations (cf. the 1930 a-paper, Crosses 1—4), but in the present cross 162 F_2 plants had a more or less clear network and only 15 individuals were of the T—A type. *The ratio 162 : 15 cannot represent a monohybrid segregation but corresponds very well with a 15 : 1 ratio,* thus indicating that the difference in question is conditioned by two polymeric factors. A comparison between the observed and expected values (165,94 : 11,06) gives a χ^2 value of 1,19 which corresponds to a P value intermediate between 0,30 and 0,20. The agreement between observation and expectation is evidently quite good. This being the case, the segregation must be assumed to involve four different factors. In such a case the theoretical ratio will be 135 : 9 : 45 : 3 : 45 : 3 : 15 : 1. Since in the present case

there were only 177 F_2 plants, three of these categories will be expected to contain less than five individuals. Under such circumstances it is not correct to calculate the χ^2 and P values for the entire segregation. However, considering each flower colour category separately, the following situation is met with. As mentioned above, there were 102 F_2 plants with pink flowers. Of these 90 had a clear network and 12 were classified as T—A types, having a quite weak network. A comparison between the observed and expected ratio (95,625 : 6,375) gives an χ^2 value of 5,30 and P value intermediate between 0,05 and 0,02. In the next flower colour category, red, all the 34 plants observed had clear network. The ratio 34 : 0, however, may correspond to a 15 : 1 segregation. According to the tables given by WARWICK (1932) the probability is 0,1114. The corresponding ratios in the white and T—D categories were 28 : 3 and 10 : 0. According to the same tables, the probability that these ratios correspond to 15 : 1 are 0,1801 and 0,5215 respectively.

Thus, though this needs to be verified by further investigation, it is rather probable that in the present cross the difference in type of network is caused by two polymeric genes, the dominant allels of which are present in the T—G line. Several other *Tetrahit* lines (T—B, T—C, T—D and T—X), giving monohybrid segregations with T—A, have only one dominant factor for clear network. Thus, the T—G line is outstanding, not only by the presence of a special factor for red flower colour (*A*) but probably also by the occurrence of two polymeric genes causing a marked network on the middle lap of the flower.

D. THE CONSTITUTION OF T—X.

According to the summary of the factorial analysis in *Galeopsis Tetrahit*, given in the 1930 a-paper (p. 202), the line T—X is known to carry the factors *a*, *H* and *I*. The following crosses revealed the presence of a fourth factor.

a. $T—C \times T—X$.

T—C represents the recessive white flower colour (with violet spots on the side lips), T—X is dominant white. Both lines have a clear network. The F_1 plants were dominant white, and in F_2 there was segregation in 39 dominant white : 10 light red : 6 recessive white (the T—C type). The segregation of red-flowered plants demonstrates that T—X, just as the line T—B, is homozygous for a red factor as well as for a completely epistatic inhibiting factor. The red factor in T—X, however, produces a lighter red colour than the corresponding factor

in the line T—B. As this light red colour is of about the same shade as in the line T—E, the red factor in T—X may provisionally be symbolized by R_1. Thus, the segregation in the present cross is dihybrid, the constitution of the parents being *HH* R_1R_1 and *hh rr*. Since HR_1 and *Hr* gives the same phenotypical effect the ratio 12 : 3 : 1 is to be expected. A comparison between the observed values (39 : 10 : 6) and those theoretically expected (41,25 : 10,31 : 3,44) shows a close correspondance. Only in the third category do we find a noteworthy difference. As in this case the number of expected individuals is lower than five, the χ^2 method cannot be used, but a calculation of the D/m value gave the figures 0,75 : 0,52 = 1,44, thus showing that this difference is not significant.

b. *T—E* × *T—X*.

As mentioned above, the red factor in T—X produced a light red colour of about the same shade as in the T—E line. In order to test whether the red factors in the two lines were different or not, an F_2 generation from T—E × T—X hybrids was raised. Unfortunately, however, only 36 F_2 plants were obtained, and of these 28 were dominant white (like T—X and the F_1 plants) and 8 had light red flowers. This ratio (28 : 8) probably represents a monohybrid segregation, which is to be expected since T—E is *hh* R_1R_1 and T—X HHR_1R_1. The number of F_2 plants, however, is not sufficient to demonstrate that the *R* factors are really identical or allelic. However, the phenotypical effect and the fact that this factor is completely hypostatic under *H* represent additional evidence that this red factor in T—X belongs to the same system of multiple allels as almost all other red factors in the *Tetrahit* lines studied.

E. THE CONSTITUTION OF T—M.

The line T—M has not been used at all in the crosses described in the 1930 a-paper. The following two crosses, however, demonstrate that this line is homozygous for the factors *a*, h_1, *R* and *I*. — T—M is characterized by red flowers of about the same intensity as in T—F. The shade of the red colour, however, is somewhat more yellowish than in most other red-flowered lines. The network is rather marked as in most *Tetrahit* types.

a. *T—M* × *T—D*.

With regard to flower colour the T—D line has the constitution *aa* h_1h_1 *rr II* (cf. the 1930 a-paper, p. 202). As expected the red colour

of T—M proved to be dominant over the faint T—D colour. In F_2 monohybrid segregation was obtained, the numbers observed being 74 red-flowered : 24 T—D plants. As no T—C plants appeared and since all F_2 plants had a network of the same type as the parents, this cross demonstrates that T—M has the constitution h_1h_1 *II* and that it also carries a red factor. Provisionally this factor may be symbolized by *R*.

b. *T—M* × *T—G*.

Phenotypical differences. — Both lines are red-flowered, but T—G is »distinct» red, in T—M the side laps are diffusely coloured.

Factorial differences. — T—G has the constitution *AA* h_1h_1 *rr*, T—M is presumably *aa* h_1h_1 *RR*.

Segregation. — The F_1 plants were diffusely red. In F_2 there was segregation in 118 red, diffuse : 37 red, distinct : 11 T—D types. The expected ratio is 12 : 3 : 1, since the *AR* and *R* categories are phenotypically indistinguishable. The agreement between the observed and the expected numbers ($124{,}_{50} : 31{,}_{13} : 10{,}_{38}$) is quite good. The χ^2 value is 1,49 and P intermediate between 0,50 and 0,30. This result shows that T—G and T—M have really different factors for red flower colour. The appearance of T—D (and no T—C) plants in F_2 is in agreement with the previous cross and represents additional evidence that T—M as well as T—G carries the factor h_1. All F_2 plants also in this cross had clear network, thus demonstrating that the lines crossed have at least one *I* factor in common.

F. MULTIPLE ALLELS FOR THE TYPE NETWORK.

The factorial basis of the type of network on the lower lip of the flower has been further elucidated by the following crosses, involving the lines T—C, T—D, T—I and T—J.

a. *T—C* × *T—J*.

Phenotypical differences. — T—C represents the recessive white colour (with violet spots on the basis of the side laps) and has a marked network on the middle lap. T—J is red-flowered and characterized by the presence of a large white spot on the side laps (MÜNTZING, 1930 a, Pl. IV, No. 10). Further, the network in T—J is almost completely lacking.

Factorial differences. — The constitution of T—J has previously been studied very little, but with regard to flower colour this line has been supposed to have the constitution *aa hh RR ii ff*. This formula is rather hypothetical and is only based on a cross with the line T—I

and on the phenotypical similarity to other lines with known constitution. The factor *j* is supposed to be responsible for the white spots on the side laps, which are lacking in most other lines. — The *T—C* line has the constitution *aa hh rr II FF*.

Segregation. — The F_1 plants were red-flowered with clear network and no spot on the side laps. In F_2 there was a segregation in 36 plants with red flowers, clear network and no spot : 7 plants with T—J flowers (red colour, faint network and white spot) : 14 plants with typical T—C flowers : 2 plants of the same type but with weak network and smaller violet spots on the side laps. The observed ratio 36 : 7 : 14 : 2 probably corresponds to an ordinary 9 : 3 : 3 : 1 segregation. On this assumption a comparison between the observed and expected numbers (33,19 : 11,06 : 11,06 : 3,69) gave an χ^2 value of 3,29 and a P value intermediate between 0,50 and 0,30. As the expected number in one of the classes is as low as 3,69 it is not quite correct to use the χ^2 method in this case, but anyhow it is obvious that the observed numbers probably represent a dihybrid segregation.

It is interesting that in this F_2 there is an absolute correlation between weak network and white spot on the side laps, and inversely, all plants with clear network had no spot. Thus it would seem that only one factor with a pleiotropic effect is responsible for the difference in question. Considering other crosses, previously described and results to be discussed below, the most probable explanation is the occurrence of three different allels, *I*, *i* and i_o, influencing the type of network. Most lines with a clear network and no spots on the side laps have the constitution *II*. According to the cross T—A × T—G the T—G line even seems to have two polymeric *I* factors. Other lines such as T—A and T—I are *ii* and have a faint network but no spots on the side laps. These spots, however, are probably only visible in red-flowered lines, and therefore it is possible that T—A upon closer examination will be found to carry i_o, the third allel in the series, and not *i*. Hitherto the factor i_o is only known with certainty in the red-flowered T—J line.

This hypothesis concerning the factorial basis of the different types of network is in the main verified by the following crosses.

b. *T—D* × *T—J*.

This cross is rather similar to the preceding cross, T—C × T—J. However, since T—D carries the factor h_1 instead of the *h* factor present in T—C, the segregation will be trihybrid. T—D has the constitution h_1h_1 *II rr* and T—J is probably *hh* i_oi_o *RR*.

Segregation. — The F_1 plants were red-flowered with clear network and no spots. In F_2 five of the six expected categories were found. Ninety-seven plants were red-flowered with clear net work and no spots, 25 plants were of the T—J type (red flowers, weak network, white spots), 22 plants had flowers of the T—D type, but three of these plants had rather weak network and faint spots on the side laps. Finally, nine plants had flowers of the T—C type. — Theoretically, the 64 combinations of the trihybrid scheme would include the following genotypical and phenotypical categories: 27 $h_1\,I\,R$ (red, clear net, no spot) : 9 $h_1\,I\,r$ (T—D flower, clear net, no spot) : 9 $h_1\,i_o\,R$ (red, T—J type) : 9 $h\,I\,R$ (red, clear net, no spot) : 3 $h_1\,i_o\,r$ (T—D flower but weak net) : 3 $h\,I\,r$ (T—C flower, clear net) : 3 $h\,i_o\,R$ (red, T—J type) : 1 $h\,i_o\,r$ (T—C flower, weak net). Adding the identical phenotypical categories, the following ratio should be obtained: 36 red, clear net, no spots : 12 T—J types : 9 T—D (clear net) : 3 T—D (weak net) : 3 T—C (clear net) : 1 T—C (weak net). According to this series the expected values per 153 will be: $86{,}_{06} : 28{,}_{69} : 21{,}_{52} : 7{,}_{17} : 7{,}_{17} : 2{,}_{39}$. The observed ratio 97 : 25 : 19 : 3 : 9 : 0 agrees rather well with the expected series. A calculation of χ^2 resulted in the values $7{,}_{44}$, the corresponding P value being intermediate between $0{,}_{20}$ and $0{,}_{10}$. As the expected number in the last category is only $2{,}_{39}$ this calculation is not quite correct. If the two last categories are added and the χ^2 of this new series is calculated, the value $4{,}_{61}$ is obtained. The corresponding P value is situated between $0{,}_{50}$ and $0{,}_{30}$. Thus, in any case the agreement between expectation and observation is sufficiently good.

c. *T—F* × *T—J.*

Further evidence that the weak network and the white spots on the side laps are pleiotropic effects of the same factor, i_o, was obtained from the cross T—F × T—J. T—F is a red-flowered line, the red colour being of about the same intensity as in T—J. In contrast to the latter line, however, T—F has a clear network and no white spots on the side laps. As expected, the F_1 plants had flowers of the same type as T—F, and in F_2 there was monohybrid segregation, 65 plants having T—F flowers and 20 individuals flowers of the T—J type. From this segregation two facts are obvious: firstly, the two T—J characters, weak net and white spots, are absolutely correlated as in the other crosses; secondly, the red factors must be allelic or even identical, since no recessive plants were segregated. The chance that 85 : 0 may correspond to a 15 : 1 segregation is less than $0{,}_{01}$.

d. *T—C* × *T—I*.

The network characteristic of the line T—I is similar to that of T—J but is slightly more marked. The main difference between the T—I and T—J flower, however, is the white spot on the side laps, which is present in T—J but absent in T—I. As described in the 1930 a-paper this difference gave monohybrid segregation and was thought to be due to a special factor pair, *F—f*. Since *f* was found to be a member of the same allelic system as *I* and *i* it was called i_o instead of *f*. As demonstrated above, i_o does not only give monohybrid segregation when crossed to a line carrying *i* but also in combination with the *I* gene, present in most *Tetrahit* lines. — The present cross, T—C × T—I, offers an opportunity of testing whether monohybrid segregation in the type of net is also obtained in the progeny of *Ii* hybrids, representing the third combination possible between *I*, *i* and i_o.

As T—C is recessive white and T—I red-flowered, the segregation in the present cross should be dihybrid, T—C corresponding to the formula *rr II* and T—I being *RR ii*. The F_1 plants were red-flowered and had a clear network like T—C. In F_2 the four expected categories appeared in the following numbers: 33 red, clear net : 19 red, weak net (T—I type) : 17 T—C type, clear net : 10 T—C, weak net. A comparison between the observed and expected numbers ($44{,}44 : 14{,}81 : 14{,}81 : 4{,}94$) gave an χ^2 value of 9,62 and a P value intermediate between 0,05 and 0,02. Thus, though there is a possibility that the observed ratio corresponds to a typical dihybrid segregation, it is more probable that some disturbing influence is at work. This is quite plausible, since the F_1 hybrids in this cross were partially sterile, the average percentage of good pollen being as low as 67. Other examples of segregation ratios disturbed by selective sterility will be discussed below. — In spite of these disturbances it is obvious, however, that segregation in this cross is dihybrid and that the factors *I* and *i* are allelic. In combination with the other crosses this result definitely shows that in the material studied there is a system of three multiple allels, $I—i—i_o$, influencing the type of the network on the lower lip of the flower.

G. THE CONSTITUTION OF A REVERSE MUTATION.

As described in detail in the 1930 a-paper (pp. 216—217), the line T—C has been in culture since 1926. During the first years of its cultivation it produced some red-flowered, obviously mutant types. Derivatives of these types have been preserved and are now present in the cultures as two red-flowered lines, which differ from the typical

T—C line mainly or exclusively in flower colour. One of these lines, however, also differs slightly in some vegetative characters. This line is derived from one of the two exceptional red-flowered plants, which appeared in a progeny of normal T—C, grown in 1929 (cf. MÜNTZING, 1930 a, p. 217, Line 11). This progeny was raised from an isolated mother plant. The plant with the deepest red colour (the intensity being about the same as in T—F) was isolated and a progeny of 35 plants obtained in the following year (1930). These plants were all red-flowered, but the intensity of the red colour was somewhat variable even in the different flowers of the same individual. This gave the impression that the amount of red colour was not quite sufficient to secure a uniform colouring of all the flowers under all environmental conditions. Dry periods seemed to be unfavourable for the development of the colour.

Since 1930 six new generations of this strain have been observed and found to behave in the same way as the family raised in 1930. The line is quite constant, like typical T—C, but is characterized by the peculiar property that the flower colour has a tendency to be slightly variable in different flowers of the same individual. This has not been observed in other »natural» red-flowered *Tetrahit* lines. As already mentioned this mutant type also differs from the typical white-flowered T—C line in some vegetative characters, e. g. leaf shape and leaf colour, possibly also height and earliness. These vegetative differences are less conspicuous than the change in flower colour, but are nevertheless quite definite. They seem to have arisen at the same time as the new flower colour but were not clearly observed until 1931, when four different progenies of this mutant type were grown. Judging from all the data available (which may be considered in detail on another occasion), the origin of this new type represents a complex mutation, affecting flower colour as well as some vegetative characters. It is especially interesting that *this mutation* — with regard to flower colour — *represents a direct transition from the recessive to the homozygous dominant stage.* As already mentioned, the progeny of the first red-flowered individual was constant, all 35 individuals being red-flowered like the mother. Also in the next generation (consisting of four progenies with 40, 12, 11 and 48 plants respectively) and later all individuals were red-flowered, though with the typical fluctuating intensity.

In order to get some information about the factorial basis of this new flower colour the mutant strain (»red T—C») was crossed to the

original white-flowered T—C line and to another red-flowered line (T—F). These crosses gave the following results.

a. *Red T—C* × *T—C.*

This cross was performed in 1930 and the F_1 hybrids were grown in the following year. As expected, the F_1 plants were red-flowered, and in F_2 there was monohybrid segregation, the values obtained being 61 red : 22 white (like typical T—C). From this ratio it is obvious that the difference in flower colour between the original and mutant type is caused by one single factor.

b. *Red T—C* × *T—F.*

The same red-flowered mutant strain was also crossed with the red-flowered line T—F, carrying the gene *R*, in order to test whether the red factors in the two types were allelic or not. The first alternative was found to be true. All the 139 F_2 plants were red-flowered like the parent lines and the F_1 plants, and no recessive plants appeared.

Thus, it is evident that the mutation from the recessive white colour to red has affected the system of multiple allels, which is responsible for the red flower colour of most red-flowered *Tetrahit* lines. More precisely expressed, the mutation involves a change from *hh* to *RR* or another red factor in the same series of allels. Whether this red factor is identical or only allelic with the *R* factor of T—F cannot be decided from the present material. Phenotypically the colour of red T—C differs from that of T—F by the fluctuating intensity in the former type. This, however, is no definite proof that the allels are different. As argued above, the mutation is most probably a complex one, also affecting other genes than *r*. This may possibly account for the peculiar colour in red T—C, but it seems more probable that this colour is caused by a new allel not present in the other red-flowered *Tetrahit* lines. — This question may be further studied by new crosses, also involving the second mutant strain obtained from T—C. This strain has also a somewhat fluctuating red colour but seems to be vegetatively identical with T—C, in contrast to the other mutant type discussed above.

H. CROSSES IN WHICH SEGREGATION IS DISTURBED BY SELECTIVE STERILITY.

In some of the crosses described above agreement between observation and expectation was not quite satisfactory, and in two cases (T—I × *glabra* and T—C × T—I) this was attributed to selective sterility. Though most F_1 generations from intraspecific crosses in

G. Tetrahit have quite good pollen fertility, some of them are partially sterile in different degrees (MÜNTZING, 1929, 1930 a, 1932 a and unpublished results). As demonstrated in detail in one particular cross, T—B × T—C, this sterility is selective, the non-functional gametes representing definite classes of gametes. If these gametes carry factors for different flower colour, the segregation ratios may be very much disturbed. The cross T—B × T—C (MÜNTZING, 1930 a, 1932 a) is a typical instance of this kind, and similar disturbances were also met with in the cross T—C × »yellow *Tetrahit*» as described in the 1932 a-paper. Since the publication of these two cases some additional crosses of the same type have been observed. The following instances may be mentioned.

a. *T—C × T—M.*

According to some crosses described above (p. 385), the line T—M has the constitution *aa* h_1h_1 *RR II*. In a cross with T—D, which corresponds to the formula *aa* h_1h_1 *rr II*, monohybrid segregation was obtained, the two lines only differing with regard to the allelic factors *R—r*. The line T—C has the constitution *aa hh rr II* and is only known to differ from T—D with regard to the factor *h*. Thus, there is a bifactorial difference between T—C and T—M, the former line being *hh rr* and T—M h_1h_1 *RR*.

The F_1 plants were red-flowered as usual, but in F_2 the expected dihybrid segregation (12 : 3 : 1) was found to be very much disturbed, the following values being obtained: 74 red : 42 T—D : 34 T—C. There is evidently a great excess in the T—D and T—C categories and too many T—C plants in comparison with the number of T—D individuals. However, the F_1 plants in this cross were partially sterile, the average pollen fertility of 14 plants examined being as low as 53 per cent good pollen. An examination of the pollen fertility in F_2 revealed significant differences between the flower colour categories, all the T—C plants being fertile, whereas in the other two categories about half of the plants were fertile, the other half partially sterile. — Though this cross has not been further studied, these results are sufficient to show that the flower colour segregation is connected with and evidently disturbed by the variation in fertility.

b. *T—C × T—L.*

This cross is quite analogous to the preceding cross, T—L having the constitution h_1h_1 *RR* like T—M. In the F_2 the following numbers were observed: 11 red : 25 T—D : 9 T—C. As in the former cross there

was a great and even more pronounced deficit of red-flowered plants and the expected 12 : 3 : 1 ratio is not realized at all. — The F_1 plants had only 58 per cent good pollen, and in F_2 the different flower colour categories were correlated with different degrees of sterility, the T—C plants being fertile, most of the red plants semi-sterile etc.

From this cross, as well as from the preceding one, some F_3 families were raised. Like the F_2 generations these were characterized by disturbed segregation ratios and correlation between flower colour and different degrees of sterility. Similar results were also obtained in other crosses. At present it seems superfluous, however, to go into further details. The examples given above suffice to show that the segregation ratios are often disturbed, if the F_1 hybrids are partially sterile. On account of this fact factorial analysis has mainly been limited to the progenies of fertile F_1 generations.

The factorial analysis in *G. Tetrahit*, described in the 1930 a-paper and the present report, is based on a total of 47 different crosses, analysed to F_2 or further generations. In six of these crosses the fertility of the F_1 generations has not been studied, and in two of the remaining crosses there is no flower colour segregation in F_2, both parents having allelic red factors. In 39 visibly segregating crosses, however, the degree of fertility in F_1 is known. Thirty of these crosses have been found to give normal segregation ratios and good agreement between observation and expectation. In nine crosses, on the other hand, the segregation has been found to be more or less disturbed. In these cases the expected categories have appeared but in more or less deviating numbers. — In order to compare the pollen fertility in the two categories of F_1 generations the following two series were made up:

Category of F_1 generations:	Percentage of good pollen: 45—50—55—60—65—70—75—80—85—90—95—100												n
Normal segregation				1	1	—	2	1	—	4	12	9	30
Disturbed »	1	1	2	1	2	—	—	—	—	1	1		9

Each variate in these series represents the average percentage of good pollen in a certain F_1 generation, and this value is based on the examination of several F_1 plants. It is striking that *the majority of the F_1 generations, giving normal segregation, have good fertility* and, inversely, *almost all the disturbed segregations were obtained from partially sterile F_1 generations.* It is obvious that the two series re-

present different distributions. This was verified by the χ^2 method (FISHER, 1930). If the series are divided in two groups with the limit situated between 80 and 85 per cent, the distribution in the first series will be 5 : 25 and in the second series 7 : 2. The expected values are 9,23 : 20,77 and 2,77 : 6,23 respectively. The χ^2 value will be 12,13, and since there is only one degree of freedom P will be much below 0,01. Thus, it is certain that the two series represent different distributions and that partially sterile F_1 generations have a stronger tendency to give abnormal segregation ratios than fertile F_1 generations. This being the case, it is evidently correct to assume a causal connection between abnormal segregation ratios and partial F_1 sterility. This was done with regard to some of the crosses discussed above.

It is true that in some cases normal segregation may be obtained from partially sterile F_1 generations and disturbed ratios from F_1 plants with apparently normal fertility. The first exceptional category is easy to understand. The haploid chromosome number of *G. Tetrahit* is 16, and many of the segregations are mono- or dihybrid. Further, since the factors (or structural differences) responsible for the sterility in a certain F_1 are probably situated in two or more chromosomes (MÜNTZING, 1929, 1930 a, 1932 a), it should indeed frequently happen that the flower colour genes are situated in other chromosomes than those responsible for the partial sterility.

It is more difficult to understand that F_1 generations with more than 90 per cent good pollen may give disturbed segregation ratios. However, in the two cases observed (T—E × *glabra* and T—L × *glabra*) the deviations from the expected numbers were not very marked and may be due to other causes (differential viability etc.) than selective sterility. On the other hand it is doubtful whether pollen fertility in F_1 hybrids is ever quite as good as in the homozygous pure lines. Recent cytological results (MÜNTZING, unpublished data) have demonstrated that slight meiotic irregularities may often occur also in F_1 plants with apparently good fertility. Thus, though the pollen may have a good and uniform appearance it is quite possible that different classes of male gametes have different viability, and in some cases this may lead to disturbed segregation ratios.

III. DISCUSSION.

Though several points have been fully considered above, some other problems remain to be further discussed. — With regard to the inheritance of hairiness the main point is the occurrence of multiple

allels in the diploid species *G. pubescens* and the observation of 15 : 1 segregations in the tetraploid species *Tetrahit*. Hitherto only four ordinary *Tetrahit* lines have been crossed with the *glabra* type. It is rather interesting, however, that in all of these crosses (or at least in three of them, in which the number of individuals is sufficiently great to allow safe conclusions) segregation was dimeric though the hairy lines represent quite different degrees of hairiness. This is evident from Fig. 2 which represents the *glabra* type (No. 1) and four hairy lines, T—F, T—D, T—I and T—L (Nos. 2—5) [1]. The T—D line (No. 3) is evidently much less hairy than T—L (No. 5) but nevertheless these lines segregated in the same way in crosses with *glabra*. It will be interesting to see if such lines as T—F (No. 2), having a very low degree of hairiness, will give the same ratio, or if possibly monohybrid segregations will be obtained.

As it is highly probable that *G. Tetrahit* represents a synthesis of the genes present in the diploid species *pubescens* and *speciosa* (MÜNTZING, 1930 a and b, 1932 b), the inheritance of hairiness in *Tetrahit* must be compared with the inheritance of the same character in *G. pubescens*. It is true, however, that the differences in the two species are not quite the same, the recessive *pubescens* line, P—C, only being devoid of bristle hairs, whereas in the *glabra* type of *Tetrahit* the smooth hairs are also absent. — Though the evidence available is yet incomplete, the genetical basis of hairiness in the two species is probably the following: —

The really »typical» *pubescens* lines have no bristle hairs but abundant smooth hairs. Probably on account of spontaneous hybridisation with the other diploid species *speciosa*, in which the bristle hairs are abundant, many or most *pubescens* lines have also different degrees of bristle hairiness (MÜNTZING, 1930 a, p. 328). That was at least the case in the present material of *pubescens* (Fig. 1). (In addition to the four *pubescens* lines described in the present paper I have five other *pubescens* types in culture. These have all different degrees of bristle hairiness. Only the P—C line is smooth.)

Thus, a minority of the *pubescens* biotypes seem to have the constitution *bb*, whereas most biotypes have different allels (B_1, B_2, B_3 . . .) for bristle hairiness. Most or all *speciosa* types have bristle hairs, but it is not known whether the factors responsible for this hairiness are identical or allelomorphic with the *B* factors in *pubescens*. Judging

[1] Of these lines all but T—F (No. 2) are involved in the present crosses. The T—E line was not available when the photo was taken.

from the quite similar effect in the two species, and considering the occurrence of spontaneous hybridisation between *speciosa* and *pubescens*, which seems to be responsible for the bristle hairiness in many *pubescens* biotypes, it is more probable that the factors in the two species are identical or allelomorphic than that they are different. — In any case the *Tetrahit* lines will contain *two* pairs of hairiness factors, if *Tetrahit* has arisen by some kind of chromosome doubling from crosses between *pubescens* and *speciosa* types, *both* of which carried factors for bristle hairiness.

Since practically only bivalents occur at meiosis in pure lines of *G. Tetrahit* and a similar situation is met with in the F_1 generations (MÜNTZING, 1930 a, 1932 a and unpublished results), it is a matter of minor importance whether the two hairiness factors are allelic or not. In any case 15 : 1 segregations will be obtained. It is true that in spite of the absence of quadrivalents intraspecific pairing might occur, which would tend to change the ratio from 15 : 1 to 35 : 1. The values actually observed, however, demonstrate that such pairing does not occur in any noticeably frequency.

According to the arguments advanced above the genes for hairiness present in *Tetrahit* are probably identical or allelic with the corresponding genes in the diploid species, *pubescens* and *speciosa*. The recessive genes present in the *glabra* line of *Tetrahit* must evidently also be situated at the same loci. However, since the *glabra* line is devoid not only of bristle hairs but also of soft hairs, it must contain genes which are still more recessive than the *b* gene present in one of the *pubescens* types. These genes may be denoted by b_o, and the *glabra* line may be supposed to have the constitution $b_o\ b_o\ b_o\ b_o$. The other *Tetrahit* lines, which are hairy, will contain four *B* factors, at least the four lines which have hitherto been tested. Since these *Tetrahit* lines represent widely different degrees of bristle hairiness, it is rather probable that the *B* factors are represented by different allels in the different lines. One line may have the constitution $B_1B_1\ B_2B_2$, another $B_1B_1\ B_3B_3$ etc. It is true that the differences in hairiness in *Tetrahit* might also be caused by other, modifying genes, but in such a case it must be assumed that these genes have no effect if the two main factors are recessive. However, if the hairiness is influenced by more than two factors it would be more natural to expect various polymeric ratios, 63 : 1, 255 : 1 etc. As only 15 : 1 ratios were obtained in spite of quite different degrees of hairiness it is more plausible that this variation is caused by multiple allels. This conclusion is highly supported by the demonstration of

such multiple allels in *G. pubescens*, one of the probable parent species from which *G. Tetrahit* once arose.

It is interesting that some other characters in *G. Tetrahit* are also controlled by duplicate genes. As already reported (MÜNTZING, 1930 a) all *Tetrahit* lines have coloured stem (presumably antocyanin), but the degree of this stem colour is very different in different lines. In some crosses (T—B × T—G, T—G × T—L and probably also T—E × T—G) plants with purely green stem were segregated, the ratio obtained being 15 coloured : 1 green. The same ratio was also obtained with regard to flower colour in several crosses, involving the line T—G. This line carries a factor for red flower colour (*A*) which is different from the red factors (R_1 and *R*) present in the other *Tetrahit* lines studied. Though having a similar effect these genes, however, are probably not identical. They react differently when combined with the factor *H* and are also associated with different types of colour distribution, the *A* factor giving »distinct» red colour, the *R*-factors »diffuse» red. It has not yet been possible to decide whether this difference is due to pleiotropism or to an absolute linkage with other genes.

Judging from the cross T—A × T—G, described above, there are also duplicate factors for the type of network on the middle lap of the flower. Though this requires further verification it is probable that the T—G line carries two dominant factors, causing a marked network, whereas the line T—A is doubly recessive. It is rather peculiar that with regard to stem colour, flower colour and type of the network the line T—G is involved in all the crosses giving 15 : 1 ratios. Thus it would seem that these ratios are caused by some property of this particular line. However, since 15 : 1 ratios were also obtained with regard to hairiness in several crosses not involving the T—G line, it is more likely that dimeric segregation ratios are a characteristic feature of the entire species and not only a peculiarity associated with some particular lines.

As was discussed in detail with regard to the hairiness, the occurrence of dimeric ratios is most probably correlated with the tetraploid chromosome number of *G. Tetrahit*, especially as the diploid species *pubescens* gave only monohybrid ratios with regard to the same character. — The possible connection between polyploidy and polymeric factors is an old problem, which is difficult to attack on account of the continuous transitions between probably identical genes with identical effects and such cases in which the same phenotypical character is influenced by quite different genes (cf. TJEBBES, 1931). However, it is

certainly not simply a chance occurrence that the most typical cases of polymeric factors have been found in polyploid species. This is true of the classical cases in wheat and oats, which were first studied by NILSSON-EHLE (1909). The occurrence of polymeric or perhaps, more correctly, homomeric genes in oats has been further investigated by other authors, especially by ÅKERMAN (1922), MEURMAN (1926) and ÅKERMAN and MÜHLOW (1933). In these papers it is demonstrated that in oats just as in wheat the maximum number of homomeric genes is three, which corresponds to the hexaploid chromosome number. A similar correlation between chromosome number and occurrence of mono-, di- and trihybrid ratios has been observed by J. CLAUSEN in *Viola* (cf. MÜNTZING, 1936, p. 358).

Besides the occurrence of duplicate genes another factorial phenomenon is of special interest in the present material, viz. the demonstration of several systems of multiple allels. Two of these systems have already been described in the 1930 a-paper and are also involved in several of the crosses discussed above. The first series is formed by the factors for red flower colour, r—R_1—R. It is rather probable that this series comprises more than these three allels, since there are certainly more than two different shades of red colour in *Tetrahit*. These differences may of course in part be caused by modifying factors, but in the closely related species, *G. bifida*, the occurrence of a similar (and possibly identical) system, comprising *four* factors (R—R_1—R_2—r) has been demonstrated (MÜNTZING, 1930 a). This makes it rather probable that more than three allels may also be found in *Tetrahit*.

The second series of multiple allels is represented by the series H—h_1—h. If the dominant inhibiting factor H is absent the plants may be either h_1h_1 or hh. If there are no red factors present, the h_1h_1 condition gives the characteristic flower colour of the line T—D and hh corresponds to the T—C colour. The factor h_1 is dominant over h.

The third series of multiple allels affects the type of the network on the lower lap of the flower. From previous results (1930 a) it was known that a weak net, such as in the line T—I, is caused by the factor i. The dominant allel of this factor, I, is responsible for the marked net present in most *Tetrahit* lines. — A third allel, i_o, situated at the same locus as I and i, has been detected by some recent crosses described above. This allel, which is present in the line T—J, causes a weak net on the middle lap as well as a white spot on the side laps. Previously, this spot was considered to be controlled by a special factor f. As this

factor was found to be allelic with *I* and *i* it may be better to call it i_o, especially since it is recessive to *I* as well as to *i*.

The fourth case of multiple allels, found in *G. pubescens*, comprises the factors b—B_1—B_2—B_3, which control the degree of bristle hairiness. As just discussed above, there is strong indirect evidence that these or analogous factors are also present in *G. Tetrahit*.

In comparison with the number of loci known (eight or nine) the number of multiple allelic systems in *G. Tetrahit* seems to be remarkably high. However, many cases of multiple allels are also known in other plant species. Like most of these cases, which have been fully discussed by STERN (1930), the multiple allels in *Galeopsis* generally influence one single character, which is represented by different intensities. That is so in the case of the red flower colour and the degree of bristle hairiness. — In the case of the network the difference between *I* and *i* is again of a quantitative nature, but the most recessive allel, i_o, also influences the colour distribution of the side laps. The series H—h_1—h, finally, is more peculiar. The *H* factor is a strong inhibiting factor, which completely suppresses the red colour produced by the factors *R* and R_1. If *H* is replaced by h_1 or h, these allels are both completely recessive to the same red factors.

The results of the factorial analysis in the *Galeopsis* species are of a rather limited interest, but the knowledge of the genetical constitution of the material is quite valuable in two respects. Firstly, the observation of disturbed segregation ratios in the progenies of partially sterile F_1 generations has greatly facilitated the analysis of intraspecific sterility in *G. Tetrahit*. Secondly, a comparison between the factorial basis of similar characters in the diploid and tetraploid species is of much interest, especially since the species in question are known to be closely related.

SUMMARY.

1) In the diploid species *Galeopsis pubescens* the degree of bristle hairiness is controlled by a system of multiple allels. The difference between red and yellow flower colour in the same species is caused by a single gene.

2) In the tetraploid species *G. Tetrahit* hairiness in four lines is conditioned by two polymeric factors. Dimeric segregation ratios were also obtained with regard to three other characters.

3) Flower colour in *G. Tetrahit* is mainly conditioned by one factor pair and three systems of multiple allels.

4) The constitution of a mutant type, representing a direct transition from the recessive to the homozygous dominant stage is discussed.

5) In several intraspecific crosses segregation is disturbed by selective sterility. Fertile F_1 generations generally give normal ratios.

Svalöf, Institute of Genetics, February 1937.

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NOTE ON A HAPLOID RYE PLANT

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IN 1922, BLAKESLEE, BELLING, FARNHAM and BERGNER reported the occurrence of a haploid mutant in *Datura stramonium*. This was the first example among flowering plants of sporophytes having half the normal number of chromosomes. Since then many new cases of the same type have been observed in other genera. These cases have been summarized e. g. by GATES and GOODWIN (1930), ROSENBERG (1930), KUHN (1930) and BLEIER (1933). As pointed out in these papers, haploid plants arise through the parthenogenetic development of haploid egg-cells. In some cases this development seems to take place spontaneously without special external influences. In most cases, however, the haploids were obtained after crossing, preferably with distantly related species, and in still other cases they were produced by treatment with low temperature. The first haploids in *Datura* were obtained in the last-mentioned way and appeared in the offspring of normal *Datura* plants, which had been exposed to low temperature at about the time of fertilisation. The haploid plants of *Crepis capillaris*, described by HOLLINGSHEAD (1928), have possibly also arisen as a result of low temperature. Judging from *Triticum monococcum*, the same effect may also be obtained by high temperature or X-ray treatment (cf. CHIZAKI, 1934). Finally, haploid sporophytes may arise as twin seedlings (cf. MÜNTZING, 1937).

In the course of a series of experiments for the purpose of obtaining chromosome doubling by temperature changes some plants of *Secale cereale* were subjected to low temperature [1]. Twenty hours after cross pollination five spikes were exposed to — 3° C. for 30 minutes. After an interval of 6 hours in the greenhouse the same treatment was repeated. One of the spikes thus treated did not produce any seeds, but from the other four spikes 1, 8, 17 and 20 seeds respectively were harvested. All of these seeds germinated in the autumn of 1935, giving a total of 46 seedlings. The majority of these young plants were quite normal. Two individuals, however, were conspicuously different and characterized by poor vigour and light green leaves. One of these

[1] For this treatment I am much indebted to Miss K. MUNDT-PETERSEN.

plants did not survive the winter, but the other one and all the normal plants wintered successfully. In the spring root-tip fixations were made (in chromacetic formalin), and the chromosome numbers determined.

All the 44 plants of normal appearance proved to have $2n = 14$ as usual, but the surviving aberrant plant was found to be a haploid, having seven chromosomes in the somatic cells (Fig. 1). Judging from the phenotypical similarity, the other aberrant plant, which died in the winter, was probably also haploid. These plants originated from different spikes, but these spikes had been treated in the same way.

It is highly probable that the haploid (or haploids) in the present case, just as in *Datura*, were produced as a result of the low temperature. — The experiment was undertaken in order to produce tetraploids, but the result obtained was just the opposite. Thus, in rye it seems to be easier to stimulate the unfertilized ovules to parthenogenetic development by cold treatment than to produce a doubling of the chromosomes in the fertilized ovule. Such a result, however, was obtained by DORSEY (1936) by subjecting the plants to high temperature. Thus, in addition to normal plants with 14 chromosomes tetraploid as well as haploid rye are now known. Also a triploid individual of the same species has been obtained by the present writer (MÜNTZING, 1937), but this plant was produced by the twin seedling method and not by temperature changes.

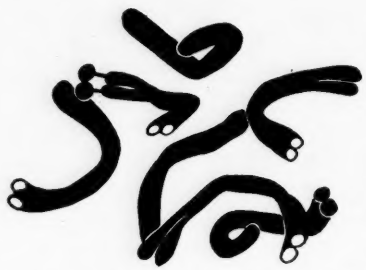

Fig. 1. Haploid rye. Somatic plate with seven chromosomes. — × 4500.

In the spring and summer of 1936 the haploid rye plant developed very slowly and finally died in spite of all efforts to keep it alive. As is evident from Fig. 2 it only reached a height of a few centimeters and did not produce any spikes before dying. The diploid sister plants, treated in the same way, developed into quite normal and vigorous plants.

The poor vigour of the haploid was certainly a consequence of the changed chromosomal constitution, and not caused by unfavourable environmental conditions. It is probably more than an incident that this unusually poor haploid was obtained in a species which is known to suffer very much from inbreeding. At least in part this inbreeding degeneration may be caused by unfavourable genes or

structural differences. In such a case most rye gametes will have »weak points» in their constitution, though the resulting zygotes may be vigorous on account of a complementary effect. The haploid rye

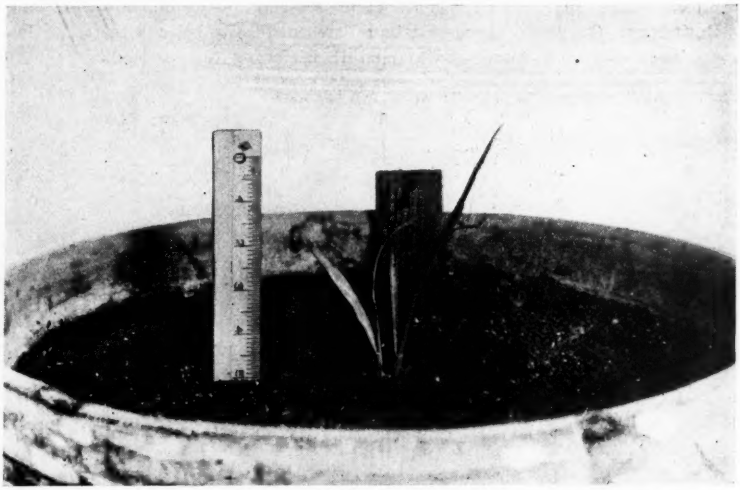

Fig. 2. Haploid rye. (When the plant died it had only reached a height of about 5 cm.).

plant was a personified sex cell. It is interesting that it revealed the poor constitution which is probably characteristic of most rye gametes.

Svalöf, Cyto-genetic Department of the Swedish Seed Association, March 1937.

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